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VOLUME 32 JUNE 1954 NUMBER 3

TULAREMIA IN BEAVERS AND MUSKRATS, WATERTON LAKES NATIONAL PARK, ALBERTA, 1952-53[1]

BY A. W. F. BANFIELD[2]

Abstract

A tularemia epizootic occurred in aquatic mammals in Waterton Lakes National Park, Alta., during the winter of 1952-53. *Pasteurella tularense* was isolated from tissues of beaver (*Castor canadensis*), muskrat (*Ondatra zibethica*), and from two water samples taken from affected streams. The course of the outbreak, including the contamination of the streams, resembled conditions described in northwestern United States by Parker, Steinhaus, Kohls, and Jellison.

Geographic Location and Extent of Epizootic

Waterton Lakes National Park is situated in the southwest corner of Alberta. It is bounded on the south by the State of Montana and on the west by the Province of British Columbia. The Park contains the break between the eastern flank of the Rocky Mountains and the foothills. The Belly and the Waterton Rivers flow north from the Park to join the Saskatchewan River system. The streams affected by the outbreak lie at elevations between 4200 and 5000 ft.

The first beaver carcass was found at the "Marquis Hole" on the Dardanelles in late September, 1952, by fishermen. Before freeze-up four more carcasses were found in the Waterton River and nearby streams. A carcass was found in a beaver pond on Crooked Creek and sent to the writer for examination on Dec. 1.

The park wardens were then instructed to search for carcasses in the beaver ponds and streams. Four additional beaver carcasses and one muskrat carcass were found in the registration office pond, and two beaver carcasses in Crooked Creek during late December and January. By that time the streams and ponds were frozen and the carcasses were found frozen in the ice.

After the spring breakup in May, the wardens again searched for beaver carcasses. Thirteen more beavers were found in Crooked Creek; one beaver and two muskrat carcasses were found in Indian Creek (a tributary of the Belly River); another muskrat was found in the registration office pond. The locations and numbers of the carcasses found are given in Fig. 1 and Table I.

[1] *Manuscript received December 2, 1953.*
Contribution of the Canadian Wildlife Service.

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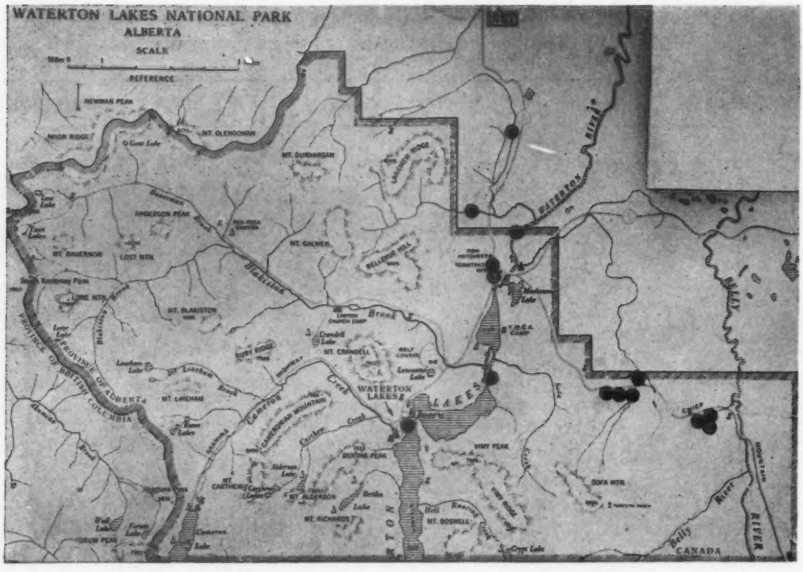

FIG. 1. Locations of carcasses found. Black circles indicate beavers and shaded circles indicate muskrats.

TABLE I

NUMBERS AND LOCATIONS OF CARCASSES FOUND

Water system	Beaver	Muskrat	Total
Waterton River	9	2	11
Crooked Creek	16	—	16
Indian Creek	1	2	3
Totals	26	4	30

Laboratory Results

The frozen beaver carcass sent to me had been partially eaten by scavengers. The thoracic cavity was intact, however, and contained dark serous fluid. The lungs were flecked with petechial hemorrhages. It was concluded therefore that the beaver had died of an acute infectious disease.

Two frozen beaver carcasses and one muskrat carcass were secured by the wardens and dispatched to the Veterinary Research Laboratory, Department of Agriculture, Lethbridge, Alta., on Jan. 6, 1953. Dr. Robert Connell of that Institution reported on Jan. 21, that cultures of *Pasteurella tularense* had been recovered from these specimens.

The post-mortem lesions noted included: excessive dark serous fluid in the body cavities, focal hemorrhages on subcutaneous fat, spleen, and lungs, liver congested and friable, spleen pale and pulpy, and gastrointestinal tract inflamed. Guinea pigs inoculated with tissue fluids from both beaver and muskrat died in five or six days. They showed typical lesions of tularemia and *Pasteurella tularense* was recovered on culture.

In order to ascertain whether the water in streams in which the beavers and muskrats had died was contaminated, three series of samples were collected during January, April, and July, 1953. A total of 32 water samples were collected as indicated in Table II. These samples were turned over to Dr. Connell. He reported on Feb. 9 that *Pasteurella tularense* had been recovered from two samples by animal inoculation and subsequent culture tests.

TABLE II

RESULTS OF LABORATORY TESTS ON WATER SAMPLES

No.	Location	Date of collection		
		Jan. 29	Apr. 3	July 5
1	Belly River Bridge	−		
2	Indian Creek cabin	−		
3	Upper Indian Creek			−
4	Crooked Creek bridge	+	−	−
5	Lower Crooked Creek			−
6	Beaver pond on Crooked Creek	−	−	
7	Beaver pond on Crooked Creek	−	−	
8	Beaver pond on Crooked Creek	−	−	−
9	Maskinonge Lake		−	
10	Fish hatchery	−	−	
11	Fish hatchery beaver pond	−	−	
12	Registration Office pond	+	−	−
13	Waterton River	−	−	
14	Hay meadow beaver pond		−	
15	Marquis hole beaver pond		−	
16	Blakiston Brook bridge		−	
17	Lower beaver pond on Blakiston Brook		−	
18	Upper beaver pond on Blakiston Brook		−	
19	Townsite dock		−	
20	Cameron Creek		−	
21	Cameron Falls		−	

\+ *Indicates positive laboratory test for Pasteurella tularense.*
− *Indicates negative laboratory test for Pasteurella tularense.*

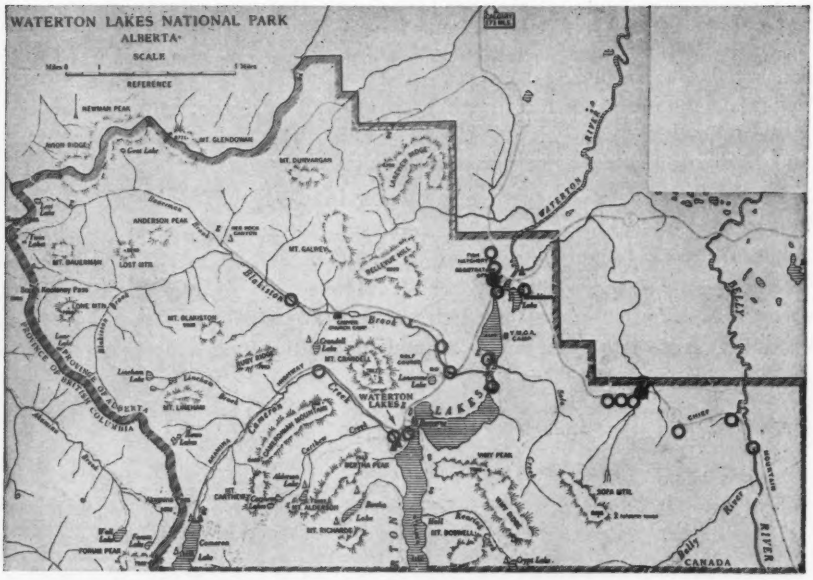

FIG. 2. Locations of water samples tested for *Pasteurella tularense*. Black circles indicate positive tests.

The locations of the water tests are shown in Fig. 2. The two contaminated samples had come from locations where a number of carcasses had been found. All subsequent water samples were negative.

Field Observations

Prior to the epizootic, the beaver population had been high. During the autumn there were few signs of recent beaver activity in many colonies. By spring the depletion of the colonies was evident. Lodges and dams were untended and the water level in the beaver ponds rapidly dropped, disclosing unutilized winter feed beds (Fig. 3). Most of the carcasses were found frozen in the pond ice, or on the dams.

In the spring the carcasses rapidly disappeared because of the plentiful scavengers. Tracks indicated that a black bear (*Ursus americanus*) had collected seven beaver carcasses from one pond and piled them on the bank where they were found. These carcasses were later consumed.

The outbreak had a mosaic effect. Beaver colonies were practically cleared from Indian Creek, Crooked Creek, and the vicinity of the Waterton River. Other colonies on Blakiston Brook appeared unaffected. By early summer there were signs of recolonization on the infected streams.

The two positive water samples came from running water below affected beaver ponds, while samples from the ponds themselves were negative.

PLATE I

FIG. 3. Abandoned beaver pond showing unutilized feed bed behind lodge at right.

Discussion

This seems to be the first time that *Pasteurella tularense* has been demonstrated in the natural waters of Canada.

The height of the epizootic seems to have been in the autumn judging by the presence of unutilized feed beds and the first discovery of carcasses. Carcasses found in the spring were decomposed. Contamination of the streams could not be demonstrated in April or July. Removal of carcasses by predators and natural drainage from abandoned beaver ponds in the spring may have led to purification of the stream waters.

There is suggestive evidence which adds support to the theory of Jellison, Kohls, Butler, and Weaver (2), that natural waters become contaminated from terrestrial mammals. It was reported that the Alberta Department of Public Health (1) collected ticks infected with *Pasteurella tularense* near Pincher Creek (30 miles north of the Park) during the summer of 1952, prior to the outbreak. During the winter a warden reported seeing an ailing snowshoe hare (*Lepus americanus*) near a pond where beavers died.

As soon as the disease had been confirmed, park wardens and the local Indian Agency were warned about the risk of contracting the disease through handling infected beavers or drinking contaminated water. There were no known human cases of tularemia associated with this epizootic.

Acknowledgments

I wish to acknowledge the co-operation of Superintendent J. A. Atkinson, Chief Warden R. Hand, and the wardens of Waterton Lakes National Park. Special attention is drawn to the co-operation of Dr. Robert Connell, and members of the Veterinary Research Laboratory, for laboratory determinations.

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STUDIES ON DIPTEROUS PARASITES OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) (LEPIDOPTERA: TORTRICIDAE)

III. *CEROMASIA AURICAUDATA* TNS. (DIPTERA: TACHINIDAE)[1]

By H. C. Coppel[2] and M. G. Maw[2]

Abstract

The tachinid parasite *Ceromasia auricaudata* Tns., which had been transferred from Western to Eastern Canada for release against the spruce budworm *Choristoneura fumiferana* (Clem.), is an insect that deposits microtype eggs on leaves which are later ingested by the host. Eggs hatch immediately after ingestion, but the parasite does not develop beyond the first larval stage until the host pupates. Then the larva develops rapidly, maturing within 10 days. The mature larva leaves the host pupal case, drops to the ground, and pupates usually within 24 hr. Nine to 11 days later, the adult emerges. Very little information is available on the overwintering habits. The life history, habits, and rearing methods are outlined and important characters of the immature stages are illustrated and described.

Introduction

The tachinid parasite *Ceromasia auricaudata* Tns. is one of the most important dipterous parasites of the spruce budworm, *Choristoneura fumiferana* (Clem.), in British Columbia. Wilkes, Coppel, and Mathers (11) and Coppel (3) listed it second and third in importance among the dipterous parasites and fourth and fifth among parasites of all orders, sometimes causing a parasitism of 13%. Dowden, Buchanan, and Carolin (6) also placed *C. auricaudata* at or near the top of the list of important parasites of the spruce budworm in Colorado, U.S.A. This parasite, restricted as it was to western North America, provided an excellent opportunity for studies in the transfer of a biotic agent within a country (i.e., from Western to Eastern Canada). Since 1943, more than 20,000 adults have been reared or propagated at the Belleville laboratory for release in Eastern Canada and the eastern United States.

Systematic Position, Distribution, and Hosts

C. auricaudata was first described by Townsend (10) in 1908, the description being limited to a record of the characters distinguishing it from *C. aurifrons* Tns. The type specimen was a female taken at Harrison, Idaho, and is now in the United States National Museum. In 1927 Curran (5) grouped three known species of *Ceromasia* under the name *Erycia rutila* (Mg.), but according to Brooks (2) *E. rutila* is a distinct species and does not occur in North America. Thus the original designation by Townsend stands.

Before release in Eastern Canada, *C. auricaudata* appeared to be restricted to western North America. Collections from British Columbia by the

[1] *Manuscript received January 11, 1954.*
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[2] *Agricultural Research Officer, Entomology Laboratory, Belleville, Ontario.*

Belleville staff were made at Lillooet, Shalalth, McGillivray Falls, Texas Creek, and Fountain Valley. Mr. G. E. Shewell (in litt.), Entomology Division, Ottawa, stated that in the Canadian National Collection there were specimens also from the following localities in British Columbia: Keremeos, Duncan, Vancouver, Emerald Lake, Oliver, Kaslo, Alberni, and Hedley. In the United States it has been collected at Pullman, Washington, and Harrison, Idaho (10); and Estes Park, Colorado (6). Dowden *et al.* (6) stated that most of the parasitic species recorded from British Columbia were also present on the eastern slopes of the Rocky Mountains in the United States.

Only two hosts of *C. auricaudata* have been recorded from field collections: the spruce budworm, *C. fumiferana*, and the fall webworm, *Hyphantria cunea* (Drury) (4).

Descriptions of Stages

Adult

The adult male of *Ceromasia auricaudata* is shown in Fig. 1. Townsend (10) described the adult as of a new species with the following characters: "Length 7 to 9 mm. Differs from *C. aurifrons* Townsend by having the anal segment wholly deep golden, same shade as parafrontal, etc.; humeri with a faint, abdomen with a more distinct golden tinge, scutellum hardly more narrowly testaceous, and thorax more distinctly vittate."

Egg

The egg is microtype, 0.2 mm. in width, 0.26 mm. in length (Fig. 2), and black in color. It is oviform or teardrop in shape, with the posterior end

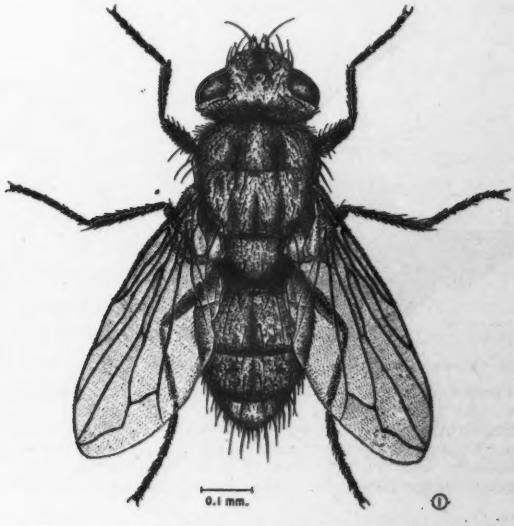
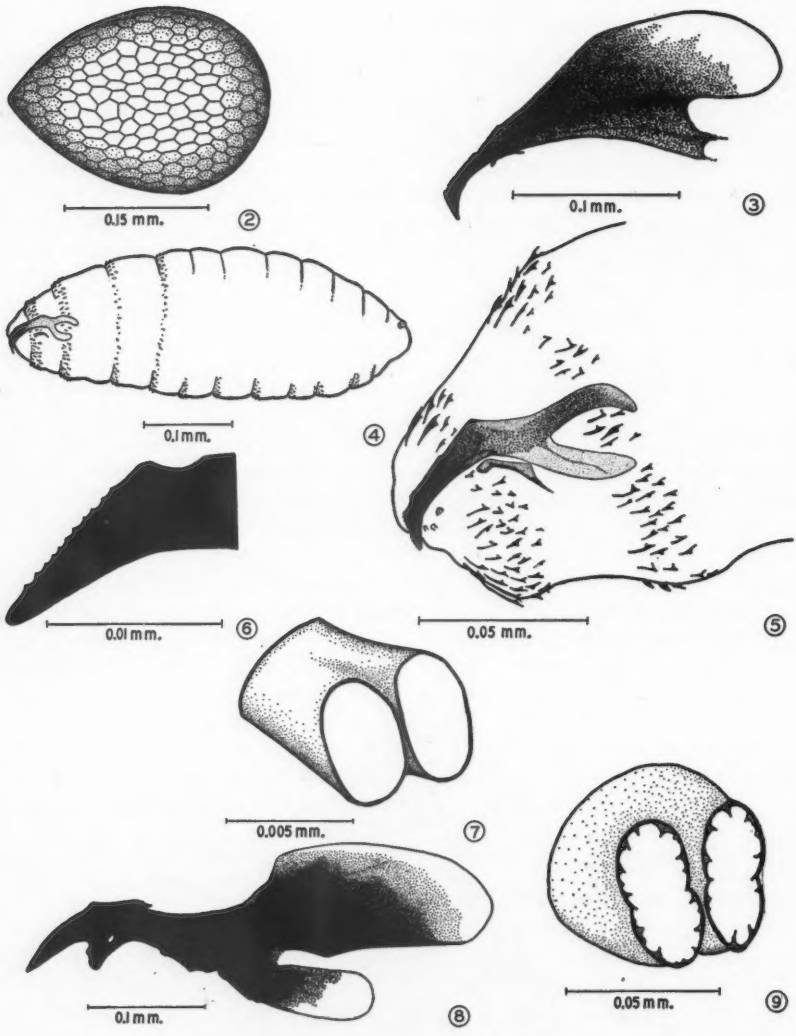


FIG. 1. *Ceromasia auricaudata* Tns., adult, male.



FIGS. 2–9. *Ceromasia auricaudata* Tns. 2. Egg. 3. Buccopharyngeal armature of first stage larva. 4. Late-first-stage larva, showing arrangement of spines, position of posterior spiracle, and the buccopharyngeal armature. 5. Anterior lateral view of early-first-stage larva, showing buccopharyngeal armature. 6. Tip of first-stage mouth hook. 7. Posterior spiracle and felt chamber of first stage larva. 8. Buccopharyngeal armature of second stage larva. 9. Posterior spiracle and felt chamber of second stage larva.

broadly rounded. It is slightly flattened on its ventral surface. Reticulations on the surface form a pattern of hexagons that can readily be seen with the aid of a microscope. No micropylar cells were recognized at the magnification used (100×), but the intrahexagonal reticulations and pigmentation are less dense at the anterior end.

First Instar

The length of the first instar larva (Fig. 4) ranges from 0.30 to 1.32 mm. The body is tachiniform and tapers markedly toward the anterior, and somewhat less toward the posterior. The integument is colorless and semitransparent, and bears conspicuous spines on the dorsal and lateral margins of the first four segments. The remaining segments have spines only on their ventral surfaces. The arrangement of the spines is shown in Table I.

In lateral view, the buccopharyngeal apparatus (Fig. 5) is highly arched in the early first instar. The apparatus is well developed and unjointed. The lateral halves converge anteriorly and unite to form a median hook that turns abruptly downward. This hook is equipped with 11 coarse teeth arranged in a row on its anterior edge (Fig. 6). The dorsal margin of the anterior part of the apparatus bears a short but moderately deep depression anteriorly, and a long, shallow depression posteriorly. The dorsal margin of the central and the posterior portion of the dorsal wings are moderately pigmented and appear in silhouette as sweeping curves. The ventral and dorsal margins of the dorsal wings are more or less parallel for most of their lengths but taper bluntly at their posterior extremities. The ventral wings are lightly pigmented. Their dorsal and ventral margins are more or less parallel and taper bluntly, much the same as the dorsal wings. The salivary plate is moderately large and pigmented. The posterior part slopes downward from the dorsal margin to form a fine, elongated point. The anterior end is club-shaped and bends downward.

In the late first instar, the buccopharyngeal apparatus is more darkly pigmented (Fig. 3), and instead of being arched in appearance the dorsal margins of the dorsal wings continue more or less straight for a distance of 0.1 mm. before they curve downward to form semitransparent blades. The dorsal and ventral wings almost lose their identities except for a moderately deep indentation in the posterior margins. The apparatus is approximately three times as large in the late first instar as in the early first instar. The pseudocephalon bears on its anterolateral surfaces the antennomaxillary complexes. These complexes include a pair of conical antennae and two pairs of rodlike sensory papillae.

The first instar larva is metapneustic. A pair of posterior stigmata (Fig. 7) are situated slightly above the longitudinal axis of the larva on its posterior segment. The stigmata are separated by a distance equal to one and one quarter times their diameter. Each stigma bears two simple openings. The felt chambers are slightly longer than broad, and are light brown in color.

TABLE I

APPROXIMATE NUMBERS OF ROWS OF SPINES ON THE DORSAL, LATERAL, AND VENTRAL REGIONS OF *C. auricaudata* LARVAE

Stage	Band	Position	Segment										
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	Anterior	Dorsal	4	3–4	3	2	0	0	0	0	0	0	0
		Lateral	1–3	2–4	2	1	0	0	0	0	0	0	0
		Ventral	4	4–5	3	2	3	2–3	2–3	2–3	2	2	0
	Posterior	Dorsal	0	0	0	0	0	0	0	0	0	0	0
		Lateral	0	0	0	0	0	0	0	0	0	0	0
		Ventral	0	0	0	0	0	0	0	1	1	2	0
II	Anterior	Dorsal	5	7–8	2–3	0	0	0	0	0	0	0	0
		Lateral	5	5	0	0	0	0	0	0	0	3–4	0
		Ventral	7–8	8–10	5–6	0	0	0	0	0	0	0	0
	Posterior	Dorsal	0	0	0	0	0	0	0	3–4	5	6–7	8
		Lateral	0	0	0	0	2–3	6	6–7	7–8	7–8	7–8	13–14
		Ventral	0	0	0	0	4	4–5	5–6	6	6–7	6–8	20
III	Anterior	Dorsal	9	8–9	7–8	5–6	5	4–5	4–6	3–4	2–3	2	0
		Lateral	1–2	8	6–7	5–6	5–6	5–6	4–6	4–6	4–5	5	0
		Ventral	9–10	7–8	6–8	6–8	7	6–8	7–8	7–9	8–9	7–8	0
	Posterior	Dorsal	0	0	0	0	0	0	0	0	0	0	5
		Lateral	0	0	0	0	0	0–1	0–1	1–2	1–3	3–4	5
		Ventral	0	0	0	0	0–1	0–2	2–3	2–3	3–4	5–6	10

Second Instar

The second instar larva has an average length of 4.56 mm., with a range of 3.62 to 5.85 mm., and resembles the first instar larva in color and form. It has a thin, semitransparent cuticle. The cuticular armature consists of short, discontinuous rows of spines. The distribution of the spines is shown in Table I. The spines are stouter, more numerous, and more darkly pigmented than those of the first instar. The 11th segment is covered with the spines except for a small area immediately surrounding the spiracular plates. The second instar larva is cradled in an envelope as described by Keilin (8). The 11th segment of the parasite larva is curved dorsally to fit into this envelope or funnel.

The buccopharyngeal apparatus (Fig. 8) is formed largely by two stout, curved mandibular hooks joined by a thin bar in the intermediate region. The apparatus is unjointed and for the most part darkly pigmented except for the posterior regions of the ventral and dorsal wings. Here, the pigmented sections merge into semitransparent blades. Immediately behind the curved portion of the hook the apparatus is greatly enlarged with a prominent ventral projection. Laterally and dorsally the enlargement appears as a distinct collar. An oval foramen is present in this region. The intermediate region broadens gradually to meet the dorsal and ventral wings. The dorsal wings begin broadly and are darkly pigmented in the anterior and central regions. The pigmentation gradually becomes less dense posteriorly and dorsally. The ventrolateral wings of the pharyngeal trough are darkly pigmented for approximately half their lengths and end in a semitransparent bladelike structure.

The pseudocephalon bears the antennomaxillary complexes as in the first instar.

As far as could be determined the larva is metapneustic. It is not known whether the lateral spiracles are functional or not, but the tracheal system is developed extensively in the pleural regions. The posterior spiracles (Fig. 9) are situated in a shallow depression above the transverse axis on the 11th segment. The spiracular plates are lightly pigmented on their posterior rims only. Each plate contains two spiracular openings, each with an irregular inner border. The felt chambers are relatively short and about one and one half times as long as broad. The two spiracular plates are separated by a distance equal to five times their diameter.

Third Instar

The length of the third instar larva ranges from 6.03 to 9.93 mm., with an average of 8.19 mm. The body tapers toward both ends, the 11th segment curving dorsally to fit into the respiratory funnel as in the second instar. The ventral surface of the body is deeply wrinkled but pseudopodia as described by Hawboldt (7) are absent. The third stage larva is much darker in color than those of the preceding stages.

The spines of the cuticular armature are broader, more nearly flat, and much less pigmented than those of the first and second instars. The spinal arrangement is shown in Table I.

The buccopharyngeal apparatus (Fig. 10) is well developed, with two distinct articulation points separating the anterior, intermediate, and posterior sections. The anterior section consists of a pair of mandibular hooks. Immediately behind the curved parts of the hooks, the dorsal and ventral margins are irregular in outline. The posterior margins of the anterior sections are indented to form two articulation sockets, into which fit the two anterior projections of the intermediate section. Near the posterior margins of the anterior sections are two mandibular foramina, one dorsad of the other. The two lateral arms of the intermediate section are connected by a ventral bridge. A small, lightly pigmented ventral plate is situated between the arms and anterior to the ventral bridge. The posterior section consists of a lightly pigmented ventral trough connecting the more darkly pigmented ventrolateral arms. The posterior tips of the arms are terminated by semitransparent blades. The dorsal wings are heavily pigmented in the basal and central regions. These dark areas are bordered anteriorly, dorsally, and posteriorly by moderately pigmented regions. Posterior to these regions is a large, semitransparent blade.

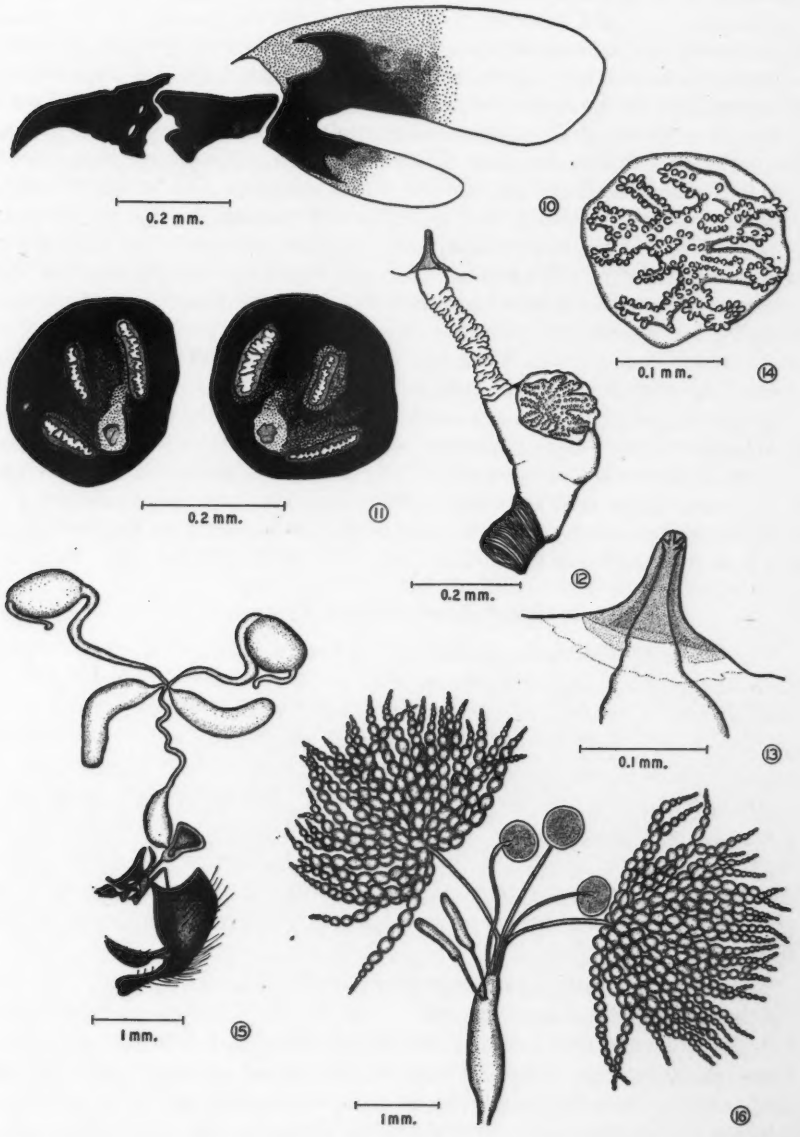
The pseudocephalon bears a pair of conspicuous antennae and two pairs of sensory organs, the ventral pair being very small.

As far as could be determined, the larva is amphipneustic although the lateral spiracles are rather prominent in some specimens. The anterior spiracles, at the posterior margin of the prothoracic segment, are well hidden and difficult to locate. The spiracles do not project above the surface of the cuticle but do come to the surface. The posterior spiracular plates (Fig. 11) are on the 11th segment above the transverse axis of the larva. The plates protrude moderately above the surrounding surface. They are black, roughly circular, and separated by a distance equal to half their diameter. The spiracular slits are three in number and are on broadly rounded ridges. The button is relatively small and is surrounded by lightly sclerotized areas that gradually become more heavily sclerotized with age.

Puparium

The puparia vary in length from 4.5 to 8.0 mm. and in width from 1.7 to 3.5 mm. The greatest diameter occurs midway between the ends. The puparium is rounded both anteriorly and posteriorly and slightly flattened ventrally. The puparium darkens with age and eventually becomes either a rich mahogany or a very dark brown in color. The remains of the spinal pattern of the third instar larva are traceable on the surface of the puparium.

The spiracular plates (Ross (9)) are on the horizontal axis of the puparium and protrude above the surrounding surface. They are shiny black, and each one bears three slits. Each slit is on the top of a broadly rounded ridge.



FIGS. 10–16. *Ceromasia auricaudata* Tns. 10. Buccopharyngeal armature of third stage larva. 11. Posterior spiracles of third stage larva. 12. Pupal respiratory apparatus. 13. Tip of prothoracic cornicle. 14. Internal spiracle. 15. Reproductive system of male. 16. Internal reproductive system of female.

The slits are irregularly bridged by lightly pigmented bars. The button is moderately large and situated ventrally near the inside edge of the plate. Immediately below the plates is a prominent elevated area with an irregular surface. This area is equal to, or slightly larger than, that of a spiracular plate but does not protrude as far from the common surface. The anal scar is oval in outline and situated on the posterior margin of the 10th segment. The anterior spiracles are often difficult to locate. They appear as a pair of small conoid papillae slightly above the transverse axis of the puparium, near the posterior margin of the prothoracic segment. The prothoracic spiracles (Fig. 12) are on the lateral surfaces of the fourth segment and appear as minute swellings. The cornicle (Fig. 13) lies almost wholly beneath the puparial wall and has at least four respiratory orifices. Each internal spiracle (Fig. 14) has 190 to 250 respiratory papillae arranged on radiating branches at the end of the atrium. Along the lateral surfaces of some of the puparia, scars of the spiracles of the mature larvae can be found.

In thin-walled puparia may be seen the two lines of cleavage at which the two halves of the puparial cap are separated from the remainder of the puparium when the adult emerges. The horizontal line extends across the front, below the anterior spiracles, and then posteriorly to the anterior margin of the fourth segment. The vertical line is less distinct than the horizontal and is at right angles to it.

Internal Reproductive Systems

The internal reproductive system of the female (Fig. 16) is a simple one. Each of the two ovaries is made up of 65 to 70 ovarioles. Each ovariole is covered with a very thin membrane and, shortly after mating, contains an average of eight fully formed eggs and many smaller egg cells. The lateral oviducts are relatively small in diameter and unite to form a common oviduct that merges into the anterior end of the uterus. The three spherical spermathecae are lightly pigmented. Each is joined to the uterus by a spermathecal duct. Behind the junction of these ducts and the uterus, the ducts of two tubular accessory glands unite with the uterus. An extensive network of tracheoles around the uterus conducts air to the developing larvae within the eggs.

The male reproductive system is shown in Fig. 15. The paired testes are lightly pigmented and ovoid in shape. At the distal end of each testis is a tail-like projection that lies along the posterior surface. The vasa deferentia have relatively large diameters near the testes and lie along the testicular surfaces for a short distance. The vasa deferentia taper gradually until they join the ejaculatory duct. At this point, two relatively large, thin-walled accessory glands join the system. Near the ejaculatory pump, the ejaculatory duct swells to a diameter approaching that of the accessory glands. The pump appears as a triangular, pigmented bulb with a darkly pigmented central area.

Life History and Habits

Methods

C. auricaudata adults were obtained from spruce budworm material received from British Columbia. The budworm material was handled in the manner described by Arthur and Coppel (1). The parasites were fed a 10% aqueous solution of honey and were sprayed twice daily with tap water. Raisins were crushed and pinned to the inside of the cages to add protein to their diet.

Observations on mating were obtained by placing one female with two or three males in a small cage (4 × 4 × 2½ in., outside measurements) for periods ranging from two to six hours daily. Since the parasite deposits microtype eggs that must be consumed by the host in order to effect parasitism, small pieces of the hosts' food were placed in the cages with newly mated females. In this manner preoviposition and oviposition data were obtained. Host larvae were starved for 24 hr. before being placed in glass vials containing small pieces of food upon which three to five eggs had been deposited. When budworm larvae were not available for these studies, substitute hosts were used. Many species were tried but *Pieris rapae* (L.), *Galleria mellonella* (L.), and *Pyrausta nubilalis* (Hbn.) proved to be the most satisfactory. *H. cunea* and *Archips cerasivorana* (Fitch) were less suitable as hosts.

Dissections of parasitized host larvae were made daily so that development of the parasites might be observed. All rearing was carried out at a day temperature of 23° C., a night temperature of 15.6° C., and a relative humidity of 60%.

Life History

Flies were frequently observed in copula in the laboratory. Females mated most readily when two, four, or six days of age but would mate at any age between 1 and 11 days. Females were not observed to mate more than once. Males would not mate when younger than two days of age but would mate at any age from 2 to 15 days. The average time to complete copulation for one lot of 50 adults under outdoor conditions varied from 55 to 95 min. A second lot of 50 adults undisturbed in the laboratory remained in copula for an average of two hours and 47 min., with a range from 30 min. to five hours.

The preoviposition period varied from 10 to 12 days. The oviposition period ranged from 3 to 26 days and averaged 12 days for 36 females studied.

The eggs are ready to hatch as soon as they are laid but must await ingestion by the host before the young larvae are freed. The eggs remain viable for at least 14 days at 23° C. and 21 days at 6°–7° C.

The parasite larva does not develop beyond the first instar until its host begins to pupate. Dissections showed living first stage larvae as late as eight weeks after eggs had been ingested by *P. nubilalis* and in no case had the larvae appeared to have done much feeding. After the host pupates, the parasite develops rapidly and spends three to four days as a second stage larva and three to four days as a third stage larva. Parasites reared on mature budworm larvae took 10.34 days from egg consumption to puparial formation.

There is evidence that when *P. rapae* larvae are used as substitute hosts late in the season the parasites may enter diapause. *P. rapae* larvae developing late in the season (September to October) normally hibernate as pupae. When these late-season larvae were parasitized by *C. auricaudata* in the laboratory, development of the parasites was prolonged even though they were held at rearing temperature (22.5° C.). Dissections of the host pupae showed the parasites in either the second or the third stage and many remained in these stages for three or four months before puparia were formed. Thus the parasites may winter as larvae in alternate hosts that hibernate in the pupal stage.

The puparium is usually completed within a 24 hr. period. Nine to 11 days after the puparium is formed, the adult emerges, the female requiring the longer period (an average of 9.36 days for 343 males and 10.29 for 365 females).

The life span of ovipositing females of *C. auricaudata* under laboratory conditions varied from 13 to 48 days, of males up to 30 days.

Excellent success was always achieved in rearing the first filial generation, the recorded sex ratio approximating 1 : 1. To date, however, this generation invariably produced male progeny only.

Habits

Laboratory Observations

Mating is not necessarily accompanied by a courtship period. The male mounts the female from behind and is rather difficult to dislodge. Slight pulsations of the body of the male may be observed from time to time during copulation.

After the preoviposition period, the female commences to lay small numbers of eggs. After two or three days the number of eggs deposited increases and reaches a peak in the midoviposition period (approximately 15 days). The number of eggs then drops rapidly and gradually tapers off until the female dies. As many as 654 eggs were deposited in a single day by one female but the average number was 64.4 per day. One lot of 56 females deposited a life average of 523 eggs per female. The largest number of eggs deposited by any one female was 1983. Eggs are deposited quickly, as many as six in 10 sec. Females may deposit eggs upon any surface but the edges and undersurfaces of foliage are apparently preferred.

Parasitism may result from ingestion of eggs during any stage of development of the host from the third to the ultimate instar. The larvae of the parasite are apparently released from the chorion by both the host's salivary juices and the mechanical action of its jaws. The parasites pass through the wall of the pharyngeal or esophageal canal. They move to the lateral longitudinal muscles on either side of the dorsal blood vessel in the host's third or fourth abdominal segment. The chorion passes through the alimentary canal and may be detected in the frass. An occasional parasite larva was found in a silk gland but never in other parts or organs of the body. The respiratory funnel may be seen readily during the advanced stages of parasite development. The external funnel opening is usually in the intersegmental membrane at the edge of the wing pad.

As the parasite larva matures, it leaves the protection of the respiratory funnel and feeds voraciously upon the body contents of the host. The mature larva escapes from its host through a rupture in the cuticula at the edges of the wing pads and in nature drops to the ground to form the puparium.

During puparial formation, the larva shortens, thickens, and assumes a rich creamy color. As the integument hardens it becomes reddish brown in color and the deep wrinkes smooth out. The puparium darkens with age and exposure to excessive moisture.

Field Observations

Adults appear in the field in British Columbia early in June, the males appearing a day or two before the females. Adults appeared at an altitude of 1000 ft. from June 18 to July 24, at 2000 ft. from July 8 to Aug. 8, and at 3000 ft. from July 11 to Aug. 9.

During the summer at Lillooet, B.C., diapause does not occur in the host or the parasite and the adults emerge as a second active generation in the field. When these adult parasites are in the field in large numbers, there are no budworm larvae present and very few larvae of any other species were found. From collections of the fall webworm, *H. cunea*, that were overwintered as pupae at the Belleville laboratory several adults of *C. auricaudata* emerged (Coppel (3)). This emergence suggests that the parasite overwinters in an alternate host and is able to deposit eggs on the foliage of either coniferous or deciduous trees.

Of particular interest in field observations in British Columbia was the abundance of *C. auricaudata* in the field after adults of the budworm emerged. It was possible, on trails near mountain springs, to collect 500 adults in an hour by inverting screened tins over the flies, which clustered on packs, clothing, etc. During eight days of collecting, only males were obtained in the first six days; in the last two days, seven mating pairs were collected but the preponderance of males was still extremely evident.

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ACCLIMATION AND LETHAL HIGH TEMPERATURES FOR A PARASITIC INSECT[1]

By W. F. Baldwin[2]

Abstract

When reared at 29° C., the parasitic insect *Dahlbominus fuscipennis* (Zett.) was more resistant to temperatures of 40° to 46° C. than when reared at 17° or 23° C. This increase in resistance was attributed to desiccation. The determination of the temperature tolerance of an insect is complicated by age, humidity conditions, and thermal history. The limits of tolerance were determined for *D. fuscipennis* reared at 17°, 23°, and 29° C. and held at temperatures of 17° to 46° C.

Introduction

The influence of temperature on the survival of insects has been investigated by many workers, and the literature contains a great deal of data concerning the ability of insects to withstand exceptionally high temperatures. In a table given by Uvarov (22), the temperatures for heat-death varied from 40° to 50° C. for various species and periods of exposure. Brues (5) reported chironomid larvae breeding in hot springs at a temperature of 49° to 51° C. Mellanby (15) lists the thermal death points of *Pediculus* sp., 46.5° C.; *Lucilia* sp., 43° C.; adults of *Xenopsylla* sp., 40.5° C.; and larvae of the latter species, 39.5° C. These temperatures are well within the range given by Uvarov. The reliability of the data is open to some question, however, since many of the early workers did not use a reliable criterion for determining the time of death, such as the 50% mortality point. Thus, their figures might be based on the time of death of either the most sensitive or the most resistant insects in their samples. Further, the upper limits of thermal tolerance have not been accurately determined for any of the species of insects studied. As recognized by Heilbrunn (12), there are very few reliable records of heat-death temperatures, since many workers have neglected the time factor.

That the range of heat tolerance of animals is subject to change by acclimation has long been recognized. The earlier work has been reviewed by Belehradek (2). In experiments with several species of flagellates, Dallinger (7) has shown that an initial exposure to 23° C. grossly affected the animals but that when the temperature was gradually raised the organisms could eventually be reared at temperatures near 70° C. Fish can also be adapted to different temperatures (10). Hathaway (11) raised the temperature of heat-death of black bass as much as 8° C. by thermal treatment. More recently, Brett (4) has shown that the lethal temperature for speckled trout can be raised by 2° C. In a later paper, Fry, Brett, and Clausen (9) delineated the

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whole field of thermal tolerance for the goldfish and demonstrated that the upper lethal temperature varies from 24° to 41° C., and that the lower lethal limit varies from below 0° to 17° C.

Several authors have presented evidence that the heat-death temperatures for insects are subject to modification by acclimation. Bodenheimer and Klein (3) found that the Palestine ant, *Messor semirufus* E. André, was more resistant to heat in July than in March. Walshe (23) showed that chironomids collected from streams below 15° C. had less resistance than individuals taken from still water at 20° C. Similarly, Whitney (24) showed that ephemerid nymphs from slow-flowing or still waters had a greater heat tolerance than those from swift streams, and he attributed the differences to greater fluctuations of temperature in undisturbed environments.

Fraenkel and Hopf (8) showed that flesh fly larvae reared at 18° C. higher than the controls had for the same exposure period lethal temperatures higher by one degree than those of larvae reared at the lower temperatures.

This paper is a report on effects of several factors, including acclimation, on the thermal limits of *Dahlbominus fuscipennis* (Zett.), an insect parasite of the European spruce sawfly, *Diprion hercyniae* (Htg.).

Materials and General Methods

The chalcid *Dahlbominus fuscipennis* was selected as an experimental animal because of the ease with which it can be reared on sawflies collected locally, the preponderance of females, and its small size. This parasite has been reared continuously at the Belleville laboratory by standardized procedures for the past 15 years. Recently its biology and ecology have been studied in some detail (Morris and Cameron (17), Ullyett (21), Reeks (20), Wilkes (25, 26), and Burnett (6)).

The regular laboratory method of propagating the parasite (Wilkes (26)) was essentially followed. Because of the recent scarcity of *Dipiron hercyniae*, cocoons of *Neodiprion lecontei* (Fitch) were used throughout. A mated female parasite was placed in a small shell vial with a sawfly cocoon, the larva in which had been paralyzed by coddling or scalding the cocoon in water at 140° F. for 1.5 min. This treatment prevented the host larva from ingesting eggs. The vials containing parasites and cocoons were plugged with cotton wool and incubated for 24 hr. at 23° C. to obtain eggs laid within daily limits. This made it possible to confine emergence of adult parasites from different groups to a one- or two-day interval. The rearing of lots of 100 vials was carried on daily for as long a period as necessary to complete a series of tests. After 24 hr. at 23° C., the females from each day's lot of vials were removed and the cocoons containing parasite eggs were placed in open vessels under controlled temperature and humidity conditions in rearing incubators. As the parasites were often delayed two or three days in escaping through the walls of the cocoons, the cocoons were opened when the developing parasites reached the black pupal stage. Two methods of obtaining samples of known age were employed in the work. In the experiments on the effects of age, the

period of emergence was confined to one hour. In all later work, the emerging adults were collected in 24-hr. lots in order to obtain large samples for the tests, and held in glass vials without food or water at suitable temperature and humidity conditions until required for experiments. Male parasites were discarded. The experimental insects were deprived of both food and water, for tests had showed that many individuals had not consumed either water or food during 24 hr.

The apparatus used in the studies consisted of three standard incubators in which the temperature and humidity could be accurately controlled, and two water baths. The temperature of the water in the baths was regulated by mercury thermostats and immersion heaters at levels from 35° to 46° C. The air supply for circulation in test chambers immersed in the baths was controlled by a simple type of air-flow regulator. The air was directed first through three 250-ml. flasks containing distilled water to bring the humidity of the air stream in the test vials as close to saturation as possible and to raise the temperature of the air to that of the bath at the same time. The air was conveyed by rubber tubing from the flasks through 6-in. vials connected in series. The vials, each containing the desired number of parasites, were fastened by clips to a wooden support and submerged in the water. The vials were then removed at intervals and placed aside for determinations of mortality at a later date. Tests to determine the temperature in the vials

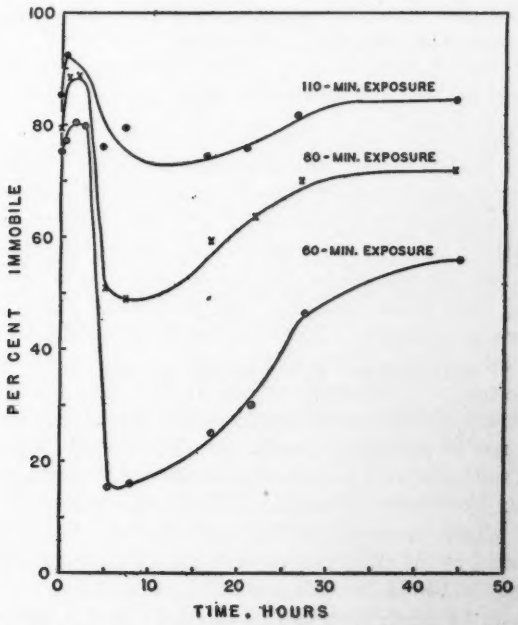

FIG. 1. Percentage of *D. fuscipennis* immobile at various times after exposure to 43° C.

showed that the change from room temperature to that of the bath was essentially instantaneous, since the glass vials were heated both by the water and by the air stream circulating through them. Unless otherwise stated, the statistical methods of analyzing the results were those of Litchfield and Wilcoxon (14).

Heat coma created a serious complication in assessing the results of early experiments. At temperatures from 40° to 46° C. the parasites became intensely active immediately after introduction to the temperature baths. This period lasted from approximately 15 min. at 46° to 60 min. at 42° C. Activity gradually decreased after these times until the insects dropped to the bottom of the vials, where they became immobile from heat stupor.

A series of tests indicated the correct time for inspection of treated samples. Three lots of 150 female parasites were subjected to 43° C. for 60, 80, and 110 min. Air was circulated through the vials at 100 cc./min. The parasites were examined with a microscope immediately upon removal from the baths and at varying intervals thereafter. The results (Fig. 1) illustrate how heat coma can hide the true results of an experiment at high temperatures. Three hours after the experiments the number of immobilized parasites was considerably greater than at five hours. Then the number of immobilized, or dead, insects gradually increased to a point where the curve flattened at 30 to 40 hr. The increase in number of dead insects from 7 to 30 hr. is attributed to delayed effects of high temperature, and it is during this period that the majority of deaths occur. Consequently, inspection of all treated samples was delayed for two days after each experiment.

Effects of Age

The initial work was seriously complicated by effects of age on resistance of the parasites to lethal high temperatures. Samples consisting of adult females emerging during a three- to four-day period contained individuals from freshly emerged to three- or four-days-old and gave highly variable results. The method adopted for obtaining insects of known age was as follows. Each day for about one month sawfly cocoons were placed with mated female parasites drawn from stock cultures. The parasitized sawfly larvae within the cocoons were incubated in a laboratory room where the temperature and humidity were controlled at 23° C. and a saturation deficiency of 8 gm./cu.m. When the parasites from each day's propagation reached the last pupal stage of development, the cocoons were opened and the parasite pupae were placed in emergence cages as previously described. When adults began to appear in some numbers, the cages were cleared of parasites and the individuals emerging during the next hour were collected. The groups were held in 4-in. vials stoppered with plastic screening until required for an experiment. The ages of the insects used in the experiments were calculated from the mid-point of the collection period to the beginning of the experiment.

Tolerance tests for adult parasites up to 96 hr. of age were conducted at 42° and 43° C. in the water baths, 20 adults being placed in each of 10 vials.

An air ventilation rate of 100 cc./min. was maintained through the vials during the experiments. The vials were removed successively at intervals during the period when mortality would occur. The times for 50% mortality, or median effective dosages (ED_{50}'s), taken from the dose–effect lines, and the confidence limits are given in Table I.

TABLE I

TIMES TO 50% MORTALITY OF *D. fuscipennis* EXPOSED TO 42° AND 43° C. AT DIFFERENT AGES (REARED AND HELD AT 23° C., AND SATURATION DEFICIENCY OF 8 GM./CU.M.)

42° C.		43° C.	
Age, in hours	ED_{50}, min.	Age, in hours	ED_{50}, min.
15	210(182–231)*	0.5	180(131–249)
25	190(181–200)	3	120(113–127)
51	145(138–152)	25	103(98–109)
74	96(87–106)	53	75(71– 79)
96	62(53– 72)	74	53(46– 61)
		95	38(33– 45)

* *95% Confidence limits.*

Table I shows that at 42° C. the times to 50% mortality decreased from 210 min. at 15 hr. of age to 62 min. at 96 hr. In the tests at 43° C., the resistance decreased from 180 min. at 0.5 hr. to 38 min. at 95 hr. of age. The results at 43° C. indicate that the most rapid change in lethal temperature occurred in the first 25 hr.

As shown by Mellanby (16) for the mosquito *Culex fatigans* Wied., exhaustion of food reserves may be a contributory cause of death at high temperatures; unfed mosquitoes died at a lower temperature than those which had been fed. Starvation would account for the decrease in resistance to temperatures of 42° and 43° C. in the present work.

Effects of Age and Acclimation

Since age is a factor in resistance of the parasite to high temperature, the interaction of acclimation and age was investigated next.

Rearing of cocoons was carried out at 17°, 23°, and 29° C., at a saturation deficiency of 5 gm./cu.m. At 17° C. the insects developed to the adult stage in 24 days; at 23° C., in 16 days; and at 29° C., in 12 days. As emergence commenced from each lot, parasites collected in 24-hr. groups were returned to the rearing incubators until time for the experiments. The collection period was extended to 24 hr. to provide large numbers of test insects. At 24, 48, 72, and 96 hr. later, insects reared and held at the three temperatures

were tested at 43° C., at a ventilation rate of 100 cc./min. The actual mean age of the insects tested at 24 hr. would thus be about 36 hr. This method, besides providing large experimental samples, made it possible to obtain parasites which had been held at the appropriate conditions over the first 24 hr. when the very rapid decrease in resistance occurs, as shown in the age experiments. Ten vials, each containing approximately 20 insects, were removed from the baths at logarithmic intervals throughout the mortality range. Each experiment was carried out in duplicate. The times to 50% mortality calculated from the provisional probit regression lines and the 95% confidence limits were:—

	24 hr.	48 hr.	72 hr.	96 hr.
29° C.	230(221–239)	210(205–216)	138(130–147)	88(76–102)
23° C.	99(72–136)	90(77–105)	86(80– 92)	63(58– 68)
17° C.	123(114–133)	108(99–118)	108(101–115)	104(94–116)

Fig. 2 shows the greater initial resistance of parasites reared at 29° C., the decline of resistance with advancing age, and the greater resistance at all ages of parasites reared at 17° than of those at 23° C.

The very high resistance of the parasites reared and held at 29° C. to a lethal high temperature showed that acclimation increased the temperature tolerance. The declining resistance of this group with age supports the finding that the age factor must be considered in lethal temperature tests.

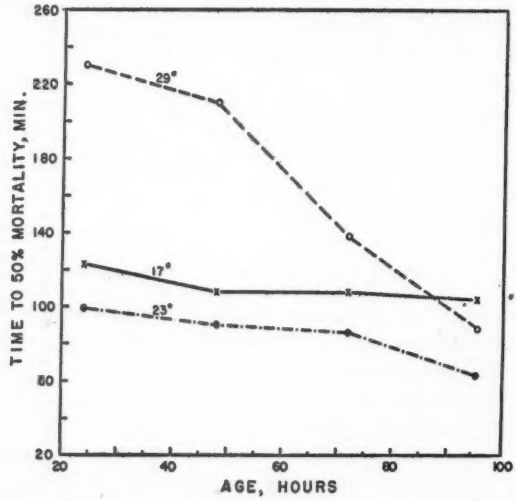

FIG. 2. Times to 50% mortality of parasites reared and held at 29°, 23°, and 17° C. (saturation deficiency 5 gm./cu.m.) and subjected to 43° C.

The inappreciable loss of resistance with age in the two groups reared and held at 17° and 23° C. was caused, no doubt, by the higher humidity conditions used for these experiments. A comparison of the curves for insects reared at 23° C. in Fig. 2 and the data given in Table I for parasites tested at 43° C. at a saturation deficiency of 8 gm./cu.m. shows the greater loss of resistance at the higher saturation deficiency.

The initial tolerance to 43° C. of parasites reared and held at 29° C. (Fig. 2) was increased to twice that of insects reared and held at 23° C. or 17° C. In the oriental cockroach, *Blatta orientalis* L. [*Periplaneta orientalis* auct.], Necheles (18) found that between 13° and 23° C. evaporation is maintained at a constant, minimal rate. Above this range of temperature the rate of evaporation is greatly increased. Therefore the rate of evaporation in *D. fuscipennis* would in all probability be constant during rearing at 17° and 23° C., whereas at higher temperatures (29° C.) evaporation would be much more rapid. Ramsay (19) has shown that an accelerated rate of diffusion at higher temperatures increases evaporation even though the saturation deficiency remains constant. It must be kept in mind that in the present study the parasites were, apart from their adult life before the high temperature tests, in an environment totally enclosed by the sawfly cocoons. The walls of the cocoon, according to Ullyett (21), have a controlling influence on the interchange of moisture between the microclimate inside the cocoon and the external environment. Ullyett's experiments were carried out at 20° C., a temperature at which evaporation, according to Necheles, would take place at a minimal rate. It is reasonable to assume that at 29° C. the loss of water by evaporation through the cocoon wall is much greater than at 20° C., and therefore the increased resistance of adults reared at 29° C. appears to be associated with a decrease in water content of the insect's body. A decrease in water content of the body cells appears also to be associated with greater resistance of parasites of all ages reared at 17° C. as compared with those reared at 23° C. At 17° and 23° C. evaporation would be at the same minimal rate, the only difference being in the time required for development of the parasite from egg to adult. At 23° C. emergence takes place in about 16 days; at 17° C., in about 24 days. The parasites developing at the lower temperature would be exposed to the same low evaporative rate for approximately eight days longer. This would result in a greater loss of water and might account for the increased resistance of parasites reared at 17° C. when exposed to 43° C. Heilbrunn (12) has presented evidence to show that desiccation increases tolerance to high temperatures.

In the same experiments (Fig. 2) the resistance of parasites reared and held as adults at 29° C. decreased rapidly with age until at four days after the emergence period their tolerance did not differ significantly from that of those reared and held at 17° and 23° C. This is clearly another example of exhausted food reserves at the high temperature of 29° C. At the lower acclimation temperatures resistance to 43° C. did not decrease markedly with advancing age since the adults were held at a saturation deficiency of 5 gm./cu.m.

Upper Lethal Temperatures

Preceding work indicated the necessity of controlling such factors as age, the amount of air passed through the vials in the high temperature baths, and both temperature and humidity during rearing. In order to determine the tolerance limits, parasites were reared at constant temperatures of 17°, 23°, and 29° C. and a saturation deficiency of 5 gm./cu.m. Samples of parasites emerging over 24 hr. were held in the incubator at the appropriate temperature and humidity for an experiment the next day. About 50 parasites were placed in each vial. The parasites from each of the three rearing temperatures were tested at one-degree intervals from 40° to 46° C. at an air rate of 50 cc./min. in the water baths. The experiments were repeated a sufficient number of times to give ED_{50} values based on a minimum of 1000 individuals (Table II).

The combined results of the experiments are shown in Fig. 3. The results have been plotted as probits against logarithmic time. Tests for parallelism proved that the lines at each lethal temperature were parallel within experimental error ($P < .05$) and could be compared by means of the "potency ratio" test (Litchfield and Wilcoxon (14)).

The times to 50% mortality (Table II), taken from the regression lines shown in Fig. 3, show the nature of the relationship between the resistances of the insects reared at the three temperatures. The ED_{50} values for the parasites reared at 29° C. were at all temperatures significantly higher than those at either of the lower rearing temperatures. Tests of the potency ratios between the parasites reared at 29° C. and those reared at 17° and 23° C. showed that the differences at all the exposure temperatures were significant

TABLE II

TIMES TO 50% MORTALITY FOR PARASITES REARED AT 17°, 23°, AND 29° C. (SATURATION DEFICIENCY 5 GM./CU.M.) AND TESTED AT TEMPERATURES FROM 40° TO 46° C.

Test temperature, ° C.	Rearing temperature					
	17° C.	No. of insects	23° C.	No. of insects	29° C.	No. of insects
40	555(512–602)*	1000	628(598–659)	1193	680(641–721)	1953
41	290(215–391)	1050	320(302–339)	2698	450(421–481)	4685
42	195(162–234)	3065	198(190–206)	4286	242(226–259)	3932
43	110(98–123)	2364	105(97–113)	4844	154(140–169)	4753
44	66(49–88)	3343	51(49–53)	3089	97(87–108)	3835
45	35(34–37)	2101	33(31–35)	3077	48(44–54)	3088
46	23(22–24)	1535	21(18–23)	1700	30(26–34)	3886

* *95% Confidence limits.*

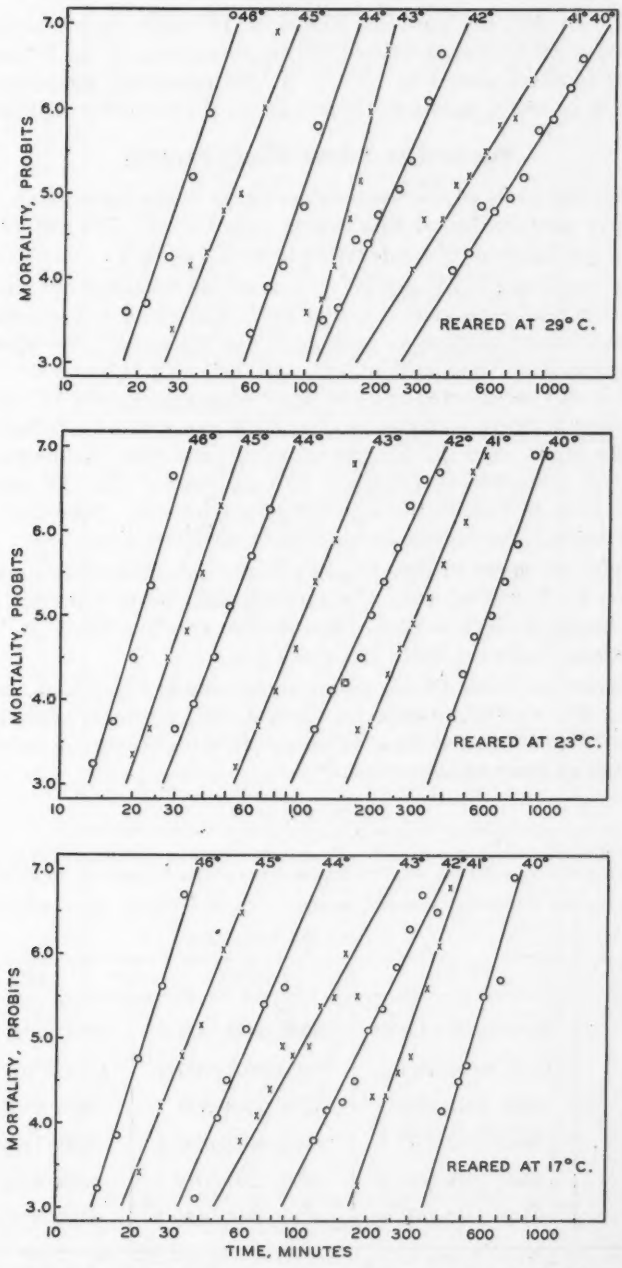

FIG. 3. Regression lines from tests with parasites reared at 29°, 23°, and 17°C. (saturation deficiency 5 gm./cu.m.) and tested at 40° to 46° C.

($P < .05$). At 44°, the parasites reared at 17° were more resistant than those reared at 23° C., but at 40° and 41° the parasites reared at 23° were more resistant than those reared at 17° C. At the remaining temperatures the resistances of parasites reared at 17° and 23° C. did not differ significantly.

Survival at Lower Temperatures

To extend the study of the temperature limits of the insect, tests at lower temperatures were conducted with insects reared at 17°, 23°, and 29° C. and held at varying intervals of temperature from 17° to 38° C.

Parasites reared at 17°, 23°, and 29° C. (saturation deficiency of 5 gm./cu.m.) were placed in 6-in. glass vials in lots of 100. The number of insects in each test was maintained as close as possible to one thousand. Parasites reared at each temperature were held at 17°, 23°, 29°, 32°, 35°, and 38° C. The number of dead were counted at 24-hr. intervals except for the 35° and 38° C. tests; at these temperatures counts were made every 12 hr. Except for the experiments at 35° and 38° C., the survival tests were conducted in the incubators at a saturation deficiency of 5 gm./cu.m. The 35° and 38° C. series were done in vials in the high temperature baths. Saturated air was circulated through the vials at 50 cc./min. in the latter tests.

The results are shown in Fig. 4 as logarithmic–probit–survival curves after the method by Litchfield (13), the survival data being expressed as lines relating cumulated effect to time. The median effective times (ET_{50}'s) and their confidence limits are listed in Table III.

The values from Table IV are shown graphically in Fig. 5 with the results obtained at 40° to 46° C. to show the times to 50% mortality over the whole range from 17° to 46° C. and the relationship of the high temperature resistance to the values at lower temperatures.

TABLE III

TIMES TO 50% MORTALITY FOR PARASITES REARED AT DIFFERENT TEMPERATURES (SATURATION DEFICIENCY 5 GM./CU.M.) AND TESTED AT TEMPERATURES FROM 17° TO 38° C.

Test temperature, ° C.	Rearing temperature		
	29° C.	23° C.	17° C.
17	21600(21239–21967)*	24000(23692–24312)	23800(23411–24133)
23	11200(11034–12376)	11800(11626–11977)	12300(12031–12472)
29	5420(5321–5520)	7720(7569–7874)	7800(7700–7901
32	5600(5512–5689)	6050(6015–6092)	7400(7296–7504
35	4000(3918–4084)	3090(2837–3257)	6640(6555–6726)
38	2540(2447–2636)	2240(2094–2386)	4780(4756–4804)

** 95% Confidence limits.*

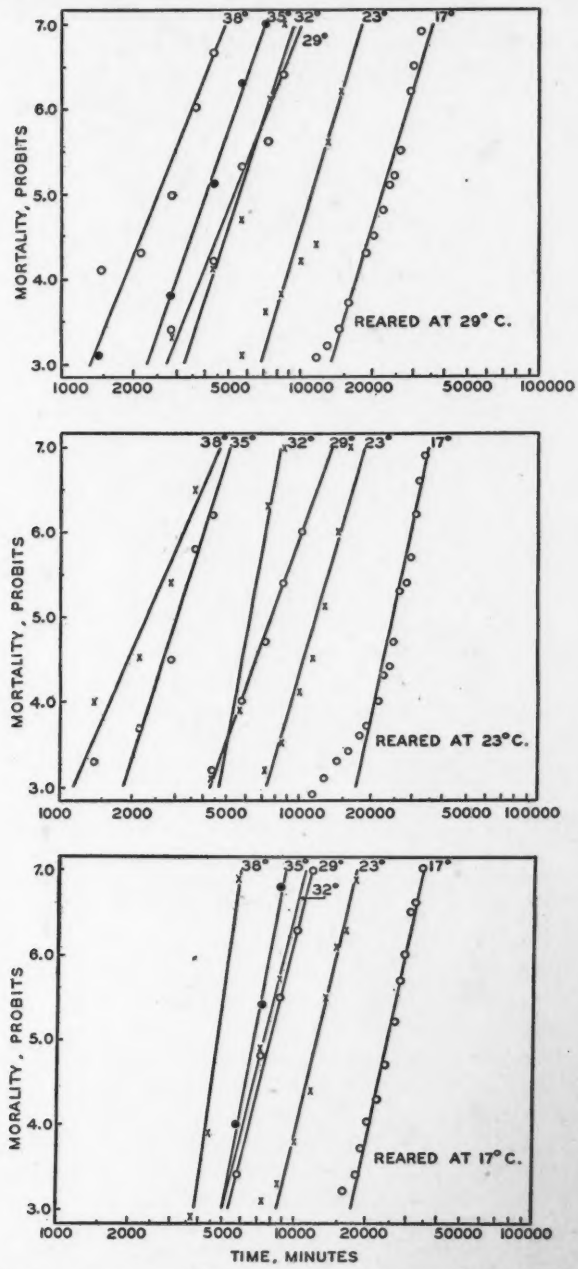

FIG. 4. Time – per cent survival curves for parasites reared at 29°, 23°, and 17° C. (saturation deficiency 5 gm./cu.m.) and tested at temperatures from 17° to 38° C.

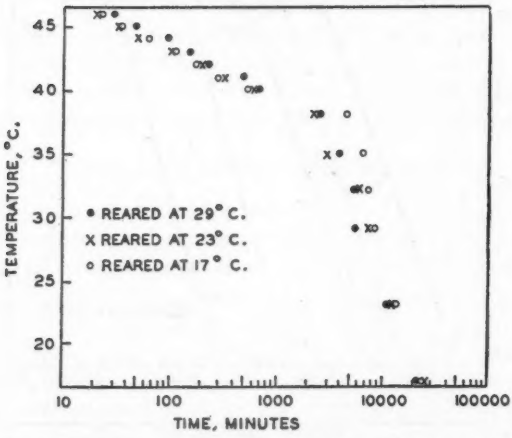

FIG. 5. Times to 50% mortality of parasites reared at 29°, 23°, and 17° C. (saturation deficiency 5 gm./cu.m.) and held at temperatures from 17° to 46° C.

Fig. 5 reveals that lines drawn through all the values above and below 38° C. would converge at a sharp angle. Above this temperature insects reared at 29° C. are more resistant than groups reared at 17° or 23° C. From 17° to 29° C. there is evidence that this relationship is reversed, the parasites reared at 29° C. surviving for a shorter period than those reared at 17° or 23° C., a result which may be explained again by Necheles' studies. The evaporation of water during rearing at 29° C. would be more rapid and the total body water at emergence would be less in comparison with those reared at 17° or 23° C. At the temperatures of 17°, 23°, and 29° C. a direct relationship between survival and amount of available water must exist, in comparison with results obtained in the range from 40° to 46° C., where desiccation increased resistance to heat.

Resistance of Adult Parasites Treated at 36° C.

The acclimation, or rearing, temperatures employed in the research to this point were confined to 29°, 23°, and 17° C. Further tests were made in treating adults to a temperature midway between 29° and 46° C. for exposure at 43° C.

The test insects were reared at 23° C. and a saturation deficiency of 5 gm./cu.m. Immediately after the 24-hr. collection period the experimental groups were exposed to 36° C. for 120 min. in vials in the water baths, saturated air being supplied at 50 cc./min. Twenty-four hours later the treated samples were tested at 43° C., untreated control lots being subjected to the same temperature. The tests were repeated at least five times.

The results are shown in Fig. 6. Straight lines were fitted to the points by means of the χ^2 test after Litchfield and Wilcoxon (14). Significant heterogeneity was found in both the experimental and the control groups, and the

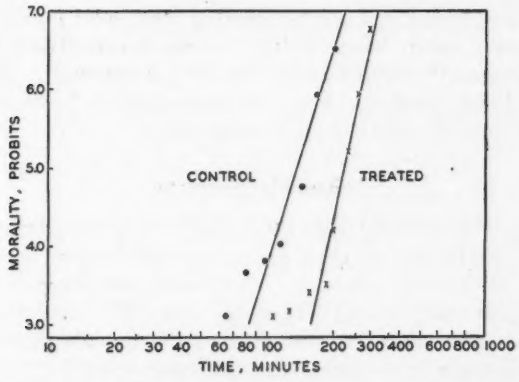

FIG. 6. Regression lines from tests with adult parasites held at 36° C. for 120 min. and exposed to 43° C.

analyses were completed by the alternate method described. The increased resistance of the treated samples is obvious in the figure; ED_{50} values calculated from the probit transformations were 220(210–231) min. for treated parasites and 134(115–155) min. for control samples.

The substantial increase in resistance of parasites reared at 23° and treated at 36° C. to a higher lethal temperature shows that the tolerance limits for adult *D. fuscipennis* can be further extended by acclimation to temperatures above the normal range.

Conclusion

The tolerance limits for insects can be altered by acclimation as shown in these studies. Experimentation with insects in a terrestrial environment, in contrast to that with aquatic animals, is complicated by several factors. One of these factors is age, the effect of which has been attributed to starvation. Humidity conditions during rearing have been shown to be important in determining the resistance of adults to high temperatures. The ventilation rate of air passed through test vials immersed in high temperature baths affected the survival of the parasites. These factors must be taken into account in designing techniques for testing temperature tolerance.

The tolerance limits were investigated over the range from 17° to 46° C. with parasites reared and held as adults at 17°, 23°, and 29° C. Subsequent work showed that treatment of adults at 36° C. for a short period of time would again extend the survival times. This fact would complicate further the accurate determination of the field of thermal tolerance.

A logical explanation of the effects of various factors on the survival of the parasites has been offered on the basis of water balance in the insect. Conditions imposed on the insects in this study which would be conducive to desiccation invariably produced increased resistance to high lethal temperatures. A suggested mode of action of desiccation on the water balance and

thermal tolerance of an insect can be found in studies by Baldwin and House (1). Treatment of sawfly larvae at high temperatures produced a significant increase in the specific gravity of the sawfly haemolymph. Recent studies have shown a direct correlation between the increase in haemolymph specific gravity and tolerance to lethal high temperatures.

Acknowledgments

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ANALYSE MICRO-CALORIMÉTRIQUE DES VARIATIONS DE LA THERMOGENÈSE CHEZ DIVERS INSECTES[1]

PAR HENRI PRAT[2]

Abstract

Experiments have been performed on various types of living materials (small vertebrates and invertebrates, bacterial cultures, germinating seeds) with a new Calvet microcalorimeter installed at the University of Montreal, thanks to a grant from the National Research Council. In this article, curves of thermogenesis (thermograms) of some species of *Melanoplus* (Orthopterae) are first examined; they vary according to individuals, species, sex, age, stage of development, temperature of the ambiance, etc. Under respiratory conditions permitting a long survival (many days), several types of normal thermogenesis can be distinguished among them. When insects are placed in closed cells, some hours later their thermograms become strongly modified; a strong paroxysm of thermogenesis occurs when the animal undergoes the first effects of asphyxia and lasts, with jerks, during two or three hours; then follows a comatous depression leading to death. Those troubles seem imputable here to accumulation of carbon dioxide, not to lack of oxygen in the calorimetric cell. In other experiments, performed in the presence of small quantities of sodium hydroxide in order to absorb the carbon dioxide elaborated, the insects remain alive for several days, with their thermograms reaching a constant, thinly undulated regimen. We have also investigated the thermogenesis of many other species of insects among Orthopterae, Dictyopterae, Lepidopterae, Dipterae, and Coleopterae. Besides specific differences their thermograms display strong variations at times of molting, metamorphosis, laying of eggs, etc. Microcalorimetry is thus likely to offer interesting applications for analyzing stages of development as well as physiological responses to environmental factors.

Introduction

La plupart des ouvrages, généraux ou spéciaux, traitant de la physiologie des insectes mentionnent les dégagements de chaleur qui, dans ce groupe, atteignent souvent des valeurs relatives considérables (1, 3, 4, 6, 10, 11). Les données énoncées à ce sujet concernent les quantités globales de chaleur dégagées pendant d'assez longs laps de temps, variant de quelques jours à quelques heures. Ces quantités de chaleur sont évaluées, soit directement par des moyens calorimétriques (5), soit indirectement à partir de la mesure des échanges respiratoires (4).

Les nouveaux micro-calorimètres Tian-Calvet (2, 12, 13) permettent de serrer le problème de plus près en fournissant un enregistrement continu de la thermogenèse. Dans ces appareils, la déviation du spot galvanométrique est proportionnelle au débit thermique à l'instant considéré, débit que l'on peut exprimer en calories par heure ou en calories par minute. Si nous enregistrons les déplacements de ce spot sur un papier photographique ou sur un ruban de papier millimétrique par l'intermédiaire d'une "photopen", nous obtenons l'enregistrement continu, minute par minute, de la thermogenèse du matériel en expérience (thermogramme) et nous pouvons suivre les moindres détails de ses fluctuations. Plusieurs publications antérieures

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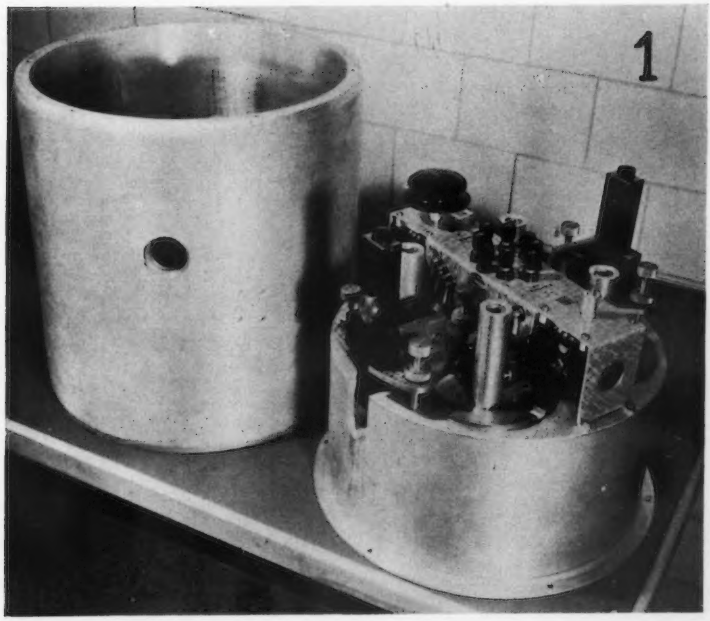

FIG. 1. Photographie de la portion centrale du micro-calorimètre Calvet et d'une de ses enveloppes métalliques: quatre blocs calorimétriques, comprenant chacun 192 thermocouples, sont branchés par paires en opposition sur deux galvanomètres. L'ensemble sera introduit dans l'enveloppe d'aluminium figurée à gauche et ensuite dans cinq autres enceintes de diamètres croissants. Chaque enveloppe métallique comporte deux fenêtres vitrées permettant aux rayons lumineux de les traverser pour se réfléchir sur les miroirs des galvanomètres. L'enveloppe externe a un diamètre de 75 cm. et une hauteur de 80 cm.

ont décrit les résultats que nous avons obtenus sur des graines en germination et sur des cultures bactériennes (7, 8, 9). Nous allons exposer ici ceux que nous ont fournis diverses espèces d'insectes. Nous décrirons en particulier quelques-unes des modifications que présente le thermogramme en fonction de l'espèce, du stade de développement, du sexe, des conditions de l'ambiance, de l'activité de l'animal, etc.

I. Appareillage

Le micro-calorimètre Calvet (2) installé en 1951 à l'Institut de Biologie de l'Université de Montréal, grâce à un octroi du Conseil National de Recherches, comporte quatre blocs calorimétriques (fig. 1), pourvus chacun de 192 thermocouples et branchés par paires en opposition sur deux galvanomètres de haute sensibilité. Dans ce nouveau modèle les enveloppes, qui entourent à la fois les quatre blocs et les galvanomètres, sont entièrement métalliques (cuivre et aluminium), avec des isolements d'amiante, ce qui élimine les inconvénients de l'humidité due aux chemises d'eau employées dans les appareils primitifs de A. Tian (12, 13). La stabilité de l'enceinte interne est d'un dix millième de degré centigrade. Chaque calorimètre possède trois sensibilités, qui sont entre elles dans le rapport de 1 à 3 et 14. Avec la plus grande, il est possible de détecter des productions de chaleur de l'ordre du cent millième de calorie.

Nous avons mentionné plus haut l'une des particularités de l'appareil, qui est de fournir directement la lecture des débits thermiques, c'est-à-dire de la production d'énergie calorifique en fonction du temps. Dans cet article nous exprimerons toujours ces débits en petites calories par heure, unités très réduites mais bien adaptées aux faibles dégagements de chaleur du matériel que nous envisageons ici. Les températures seront toujours données en degrés centigrades.

Nous avons utilisé concurremment deux modes d'enregistrement en projetant le rayon lumineux provenant du galvanomètre soit sur un papier photographique recouvrant un tambour tournant, soit sur un chariot mobile pourvu de deux cellules photo-électriques, chariot accompagnant les déplacements du rayon et entraînant une plume qui inscrit un trait continu sur un ruban de papier millimétré à déroulement régulier (système "Photopen" de Beckman). Les deux systèmes fournissent des courbes en tous points comparables, sauf quelques menus détails dus à l'inertie différente des enregistreurs. Le second offre l'avantage de rendre apparent à chaque instant le débit thermique, ce qui permet par exemple d'observer immédiatement les réactions d'un animal soumis à une influence toxique. Avec le premier dispositif, il fallait attendre le développement du papier pour connaître les résultats de l'expérience; on ne pouvait donc modifier le processus expérimental en cours de route selon les réactions du matériel. La plupart des figures fournies dans cet article sont des photographies de thermogrammes obtenus par le premier procédé, c'est-à-dire sur papier photographique. Cependant, pour comparaison, nous avons donné sur les figures 18 et 23 les photographies de deux enregistrements par "photopen".

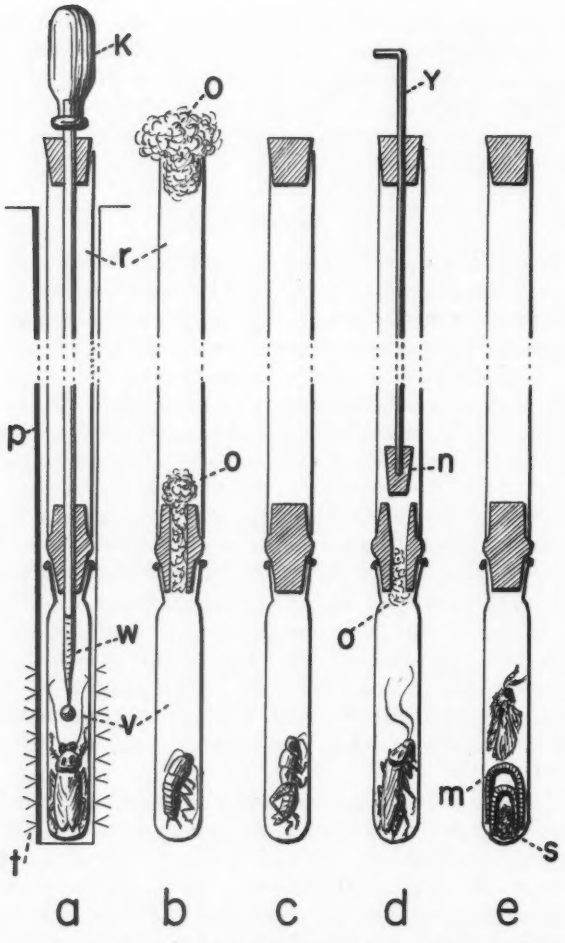

FIG. 2. Divers types de cellules (*v*) employées pour les expériences micro-calorimétriques sur matériels vivants: *a*: montage permettant l'introduction de liquide (*w*) en cours d'expérience (par exemple d'eau ou de toxiques pour enregistrer les réactions d'un animal); *t*: thermocouples; *k*: poire en caoutchouc; *p*: puits calorimétrique; *r*: tube de verre. Les bouchons, en liège ou en caoutchouc, sont figurés en hachures. *b*: Montage permettant la respiration par diffusion à travers un tampon d'ouate (*o*). *c*: Expérience en conditions asphyxiques: tube fermé, sans absorption du gaz carbonique. *d*: Disposition permettant de passer de la respiration par diffusion du mode (*b*) aux conditions asphyxiques du type (*c*) en cours d'expérience: un bouchon (*n*), mû par une tige (*y*) vient obturer, à l'instant voulu, l'ouverture de la cellule. *e*: Dispositif éliminant l'anhydride carbonique au moyen de laine de verre imbibée de soude (*S*); deux toiles métalliques (*m*) empêchent l'animal d'entrer en contact avec l'alcali.

La figure 2 montre quelques-uns des dispositifs que nous avons employés pour l'étude de la thermogenèse des insectes, des micro-organismes et des plantules. Les cellules de verre utilisées (*v*) ont un diamètre de 18 mm. et

une hauteur de 105 ou de 125 mm. Pour les mettre en place au fond des puits calorimétriques (*p*), elles sont prolongées par un tube de verre (*r*), de même diamètre et de 50 cm. de longueur. Une fois en place, elles se trouvent ainsi entourées par les 192 thermocouples (*t*), disposés en couronnes superposées. En *a*, nous avons figuré un dispositif qui permet d'introduire un liquide (*w*), au cours de l'expérience, en agissant sur une poire de caoutchouc (*K*). La perturbation thermique ainsi produite ne dure qu'un temps très court: quelques minutes; elle peut donc être aisément distinguée des phénomènes biologiques étudiés, qui se développent à un rythme beaucoup plus lent, durant plusieurs heures. Le liquide peut être de l'eau, ce qui est le cas lorsqu'on étudie la thermogenèse des germinations (7, 9), ou bien un toxique ou un anesthésique, comme lorsqu'on observe les réactions d'un insecte à divers agents chimiques. Sur les dessins *b* à *e*, nous n'avons représenté que la cellule et le tube qui la surmonte, sans répéter la figuration du puits calorimétriques ni des thermocouples.

Le dessin *b* montre un des dispositifs que nous avons le plus couramment employés pour l'étude de la thermogenèse des insectes. Un tampon d'ouate (*o*) assure un isolement thermique satisfaisant tout en permettant une suffisante diffusion des gaz. Dans ces conditions un animal peut vivre dans la cellule pendant plusieurs jours sans donner de signes d'asphyxie. En *c*, au contraire, la cellule est complètement close et l'asphyxie se manifeste au bout de quelques heures. En *d* est représenté un dispositif permettant de pratiquer successivement des expériences des types *b* et *c* sur le même sujet. D'abord la respiration peut s'effectuer par diffusion à travers le tampon d'ouate *o*; puis, à un moment donné, on peut obturer complètement la cellule au moyen du bouchon *n*, mû par une tige métallique *y*; le changement de régime du thermogramme se manifeste ainsi à partir d'un instant bien déterminé.

En *e*, pour éviter l'asphyxie en dépit de la fermeture de la cellule, on place dans le fond de cette dernière un peu de laine de verre (*s*) imbibée d'une solution de soude caustique à 20%. On interpose ensuite deux petites pièces de toile métallique fine (*m*) pour empêcher l'animal en expérience d'entrer en contact avec l'alcali. Nous verrons plus loin que, dans ces conditions, l'animal peut survivre beaucoup plus longtemps que dans les expériences de type *c*.

Nous devons souligner, fait important pour l'interprétation du comportement des animaux, que toutes les expériences décrites ci-dessous ont été effectuées dans l'obscurité totale. Nous préparons en ce moment des dispositifs qui permettront de les répéter en fournissant aux cellules calorimétriques un éclairage axial d'intensité et de composition réglables.

La figure 3 montre quelques-uns des types de tracés obtenus avec les dispositifs *b* et *e*. Nous allons examiner les modalités de leur manifestation en prenant comme exemples diverses espèces d'orthoptères appartenant au genre *Melanoplus*.

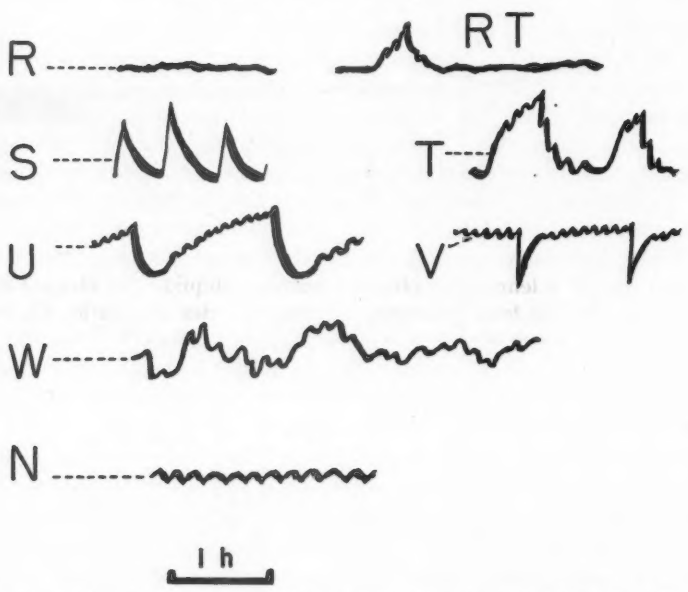

FIG. 3. Quelques-uns des types de thermogenèse observables chez les insectes étudiés: *R*: type quiescent; *S*: montées abruptes, en dents de scie; *T*: montées brisées; *U*: montées progressives; *V*: chutes brusques suivies de remontées rapides et de paliers ondulés; *W*: type irrégulier; *N*: type finement ondulé, obtenu en présence de soude (dispositif *e*). *RT*: combinaison des types *R* et *T*.

II. Thermogrammes de *Melanoplus*

Les figures 4 à 9 réunissent les photographies de six thermogrammes de *Melanoplus* Stäl (Catantopidés, Orthoptères), obtenus à 24.9° C., avec le dispositif représenté en *b*, fig. 2. La respiration pouvant s'opérer par diffusion des gaz à travers le tampon d'ouate, l'animal demeure en bonne forme pendant plusieurs jours, en dépit de sa réclusion; à la fin de l'expérience, il témoigne encore d'une vitalité parfaitement normale.

Dans l'interprétation des résultats nous aurons à considérer, d'une part, les débits thermiques absolus, tels qu'ils apparaissent en ordonnées sur les thermogrammes, et les débits thermiques relatifs, ramenés à une masse d'un gramme de l'animal en expérience. Pour des raisons évidentes (impossibilité d'évaluer avec précision la surface de l'animal) nous n'avons pas

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FIGS 4 à 9. Photographies de thermogrammes de diverses espèces de *Melanoplus*. Individus adultes, à 24.9° C., respirant par diffusion (dispositif *b*, fig. 2). Les temps, exprimés en heures, sont portés en abscisses; les débits thermiques absolus, exprimés en calories-heures, en ordonnées. FIG. 4. *M. differentialis* mâle, pesant 0.797 gr. Régime de thermogenèse de type *RTS*. FIG. 5. *M. bivittatus* femelle, de 1.312 gr.; type *S*. FIG. 6. *M. bivittatus* mâle, de 0.591 gr. L'expérience ayant duré 48 h. le thermogramme montre la superposition de deux tracés; l'horaire des paroxysmes à partir du début de l'observation est indiqué. Type *RT*. FIG. 7. *M. femur rubrum*, mâle, de 0.207 gr.; type *W*. FIG. 8. *M. mexicanus mexicanus* mâle de 0.202 gr.; type *U*. FIG. 9. *M. mexicanus atlanis*, mâle, de 0.185 gr.; type *RS*.

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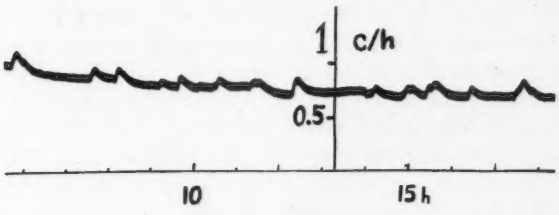
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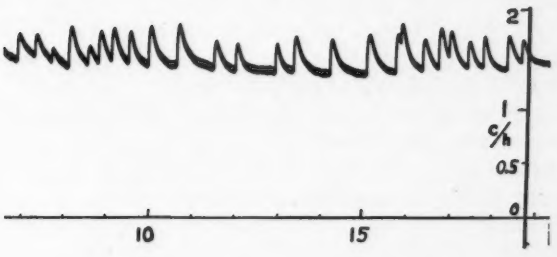
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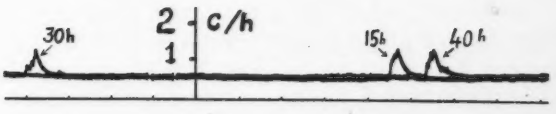
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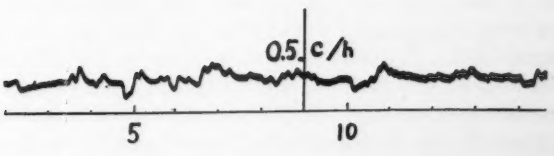
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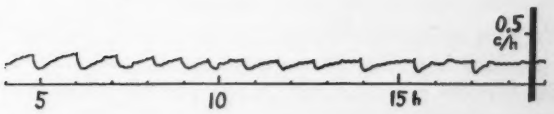
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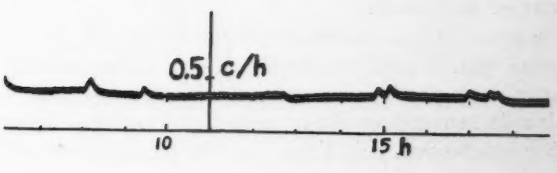
0.5 c/h
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15 h

cherché à évaluer les débits thermiques relatifs en fonction de la surface ainsi qu'on le fait en général dans les mesures de métabolisme.

La figure 4 est l'enregistrement fourni, dans les conditions indiquées plus haut, par un mâle adulte de *M. differentialis* (Thomas), pesant 0.797 gr. On y observe des accroissements passagers du débit thermique dus à des crises d'agitation et séparés par des phases de repos. Pendant ces dernières, le débit thermique tombe à sa valeur minimum qui est égale, en fin d'expérience, à 0.9 cal. h. par gramme d'animal. Les débits maxima au début de l'expérience, atteignent des valeurs de 1.6 cal. h. par gramme.

La figure 5 est le thermogramme d'une femelle de *Melanoplus bivittatus* (Say) pesant 1.312 gr. On peut y noter des oscillations fortes et assez régulières de la thermogenèse, avec une période moyenne de 30 m., entre des valeurs de 1 et 1.4 cal. h. par gramme.

Le thermogramme 6, fourni par un mâle de la même espèce, pesant 0.591 gr., montre une allure toute différente. Les paroxysmes de thermogenèse dus à des crises d'agitation sont ici séparés par de longs intervalles de repos, atteignant 10 h. en moyenne. Sur cette figure, l'expérience ayant duré 48 h., on voit la superposition de deux tracés; pour les distinguer nous avons indiqué sur chacun l'époque des paroxysmes, en heures à partir du début de la mise en cellule. Pendant les longues phases de repos, la production de chaleur tombe à 0.9 cal. h. par gramme, mais pendant les phases d'agitation, elle s'élève jusqu'à 2.4 cal. h. par gramme, d'où un rapport M/m entre les extrêmes égal à 2.4.

Les thermogrammes 7, 8, 9 se rapportent respectivement à un *Melanoplus femur rubrum* (DeGeer), un *M. mexicanus mexicanus* (Saussure), et un *M. mexicanus atlanis* (Riley), tous trois des individus mâles.

Dans le tableau I, nous avons rassemblé les données numériques fournies par ces enregistrements et par quelques autres qui n'ont pas été figurés sur les figures 4 à 9. Dans la 7e colonne nous avons indiqué le rapport M/m entre les maxima et les minima du débit thermique. Chez tous les *Melanoplus* adultes examinés, ce rapport est demeuré compris entre 1.3 et 2.7.

Ces exemples permettent de dégager, en première analyse, six types principaux de thermogenèse, que nous avons désignés par des lettres sur la figure 3 et dans la dernière colonne des tableaux:

1. Un type *quiescent R* (fig. 6 et *R*, fig. 3) comporte de longues périodes de repos pendant lesquelles la thermogenèse est très régulière et plutôt basse, périodes coupées par de rares paroxysmes qui peuvent être rattachés à l'un des types suivants, généralement au type *T*. Chez *Melanoplus*, nous avons observé le type *R* uniquement sur des mâles et dans les espèces: *differentialis*, *bivittatus* et *mexicanus*.

2. Le second type, à *montées abruptes* ou en *dents de scie* (*S*, fig. 3), est représenté par le thermogramme 5. Il comporte des oscillations assez régulières sans phases de repos. A chaque fois, on observe une montée soudaine de la thermogenèse, atteignant d'un jet le maximum, suivie d'une descente progressive, puis d'une nouvelle montée brusque. Chez *Melanoplus*

TABLEAU I

THERMOGENÈSE DE *Melanoplus* À 24.9° C.

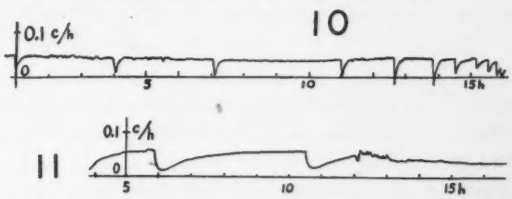
Espèce	Sexe	Poids en grammes	Courbes des figs. 4 à 9	Thermogenèse relative en cal./h. pour 1 gr.		Rapport, M/m	Type
				minimale (m)	maximale (M)		
differentialis (Thomas)	M	0.797	4	0.9	1.4	1.5	RT
	M	1.083	—	0.8	2	2.5	R
	F	1.524	—	0.9	1.3	1.4	W
bivittatus (Say)	F	1.312	5	1	1.4	1.4	S
	M	0.591	6	0.9	2.2	2.4	R
femur rubrum (De Geer)	M	0.207	7	1	2	2	W
	F	0.211	—	1	2.4	2.4	W
mexicanus mexicanus (Saussure)	M	0.202	8	0.5	1.2	2.4	U
	M	0.252	—	1.2	2.5	2	R
mexicanus atlanis (Riley)	M	0.185	9	1.6	2.7	1.7	RT

nous l'avons observé seulement sur des femelles, et dans les espèces *differentialis* et *bivittatus*.

3. Un troisième type, à montées brisées (*T*, fig. 3), se distingue du précédent en ce que la phase de montée de la thermogenèse n'atteint pas d'un seul coup le maximum; elle n'y parvient qu'après un certain nombre de brisures de la courbe (fig. 6).

4. Un autre type, que l'on peut appeler *progressif* (*U*, fig. 3), comporte également des oscillations régulières, mais leur régime est exactement l'opposé du type *S*. D'abord une montée progressive, ondulée, du débit thermique; puis une descente brusque. Nous l'avons rencontré chez les jeunes de *M. differentialis* et chez *M. mexicanus mexicanus* (fig. 8).

5. Un type à *descentes et montées brusques V* s'en distingue par le fait que les parties concaves de la courbe affectent la forme d'un *V* et non plus d'un *U* comme dans le cas précédent. La montée est presque aussi brusque que la descente. En dehors de ces fléchissements, courts et profonds, le thermogramme est très régulier, présentant une sorte de plateau ondulé (fig. 10).



FIGS 10 et 11. Photographies de thermogrammes de nymphes de *Melanoplus differentialis* à 31.4°, respirant par diffusion: FIG. 10: jeune nymphe vermiforme, 5 jours après l'éclosion, poids: 0.004 gr.; type *V*, FIG. 11: nymphe après la 3ème mue; 0.013 gr. type *U*.

6. Un dernier type, *irrégulier* (*W*, fig. 3), représenté par la courbe 7, comporte des variations irrégulières de la thermogenèse. Nous l'avons rencontré chez des individus appartenant à la plupart des espèces étudiés, sauf *mexicanus mexicanus*. Il est spécialement répandu chez les larves molles. Entre ces types principaux de thermogenèse, des intermédiaires peuvent être observés, que nous désignerons par des combinaisons de lettres: *RT*, *RS* (fig. 6), etc. D'autres peuvent apparaître seulement dans certaines conditions expérimentales, comme nous le verrons plus loin pour le type *N*.

Les thermogrammes des figures 10 et 11 ont été fournis par des individus immatures de *Melanoplus* à 31. 4°; il est facile d'observer les différences qui les séparent de ceux des adultes figurés sur les figures 4 à 9. Le thermogramme 10 est celui d'une jeune nymphe vermiforme cinq jours après son éclosion. Malgré son très faible poids, 4 mg., elle fournit une thermogenèse appréciable, s'élevant jusqu'à des maxima de 0.04 cal. h. en valeur absolue, soit des valeurs relatives de 10 cal. h., c'est-à-dire beaucoup plus que les adultes. Le mode de thermogenèse appartient ici au type *V* (fig. 3); il est d'abord très régulier, avec une chute toutes les trois ou quatre heures. Puis, après 12 h. de réclusion dans la cellule calorimétrique, le thermogramme devient de plus en plus accidenté; les phases de dépression se rapprochent et on obtient finalement un type irrégulier *W*, jusqu'à la mort, qui survient au bout de 18 h.

Le thermogramme de la figure 11 est fourni par une nymphe plus âgée, ayant accompli sa troisième mue et atteignant un poids de 13 mg. On voit ici réalisé le type *U* de thermogenèse, qui demeure au début assez régulier. Puis, après 12 h., des irrégularités se manifestent, suivies d'une dépression. Au moment des maxima, le débit thermique atteint 0.05 cal. h. en valeur absolue, soit des valeurs relatives de l'ordre de 8 cal. h. par gramme, ce qui est encore très élevé. Au moment des minima la thermogenèse peut, chez les formes larvaires, devenir nulle. Parfois même l'appareil enregistre, comme on peut le voir sur la figure 10, des valeurs négatives. Il semble possible d'expliquer ces dernières par des faits d'évaporation.

Nous avons rassemblé dans le tableau II quelques données relatives à des individus larvaires et adultes de *Melanoplus differentialis*, à 31.4°. Dans les deux premières semaines après l'éclosion, la thermogenèse est du type *V*; à partir de la seconde mue, elle passe au type *U*, puis *W*. Elle peut évoluer ensuite vers le type *R* chez les individus mâles, ou *S* chez les femelles. Toutefois, certains individus adultes, surtout parmi les femelles, peuvent conserver le type irrégulier *W*.

Au point de vue des débits thermiques relatifs, c'est-à-dire ramenés à un gramme, les jeunes offrent en général des minima plus faibles et des maxima plus élevés que les adultes.

Si l'on compare les adultes entre eux, on voit que les individus de faible poids ont une thermogenèse proportionnellement plus forte que les individus plus lourds, mais cette relation n'est pas absolue, les variations individuelles pouvant l'inverser, ainsi que nous l'avons mentionné plus haut.

TABLEAU II

THERMOGENÈSE DE *Melanoplus differentialis* (THOMAS) À 31.4° C.

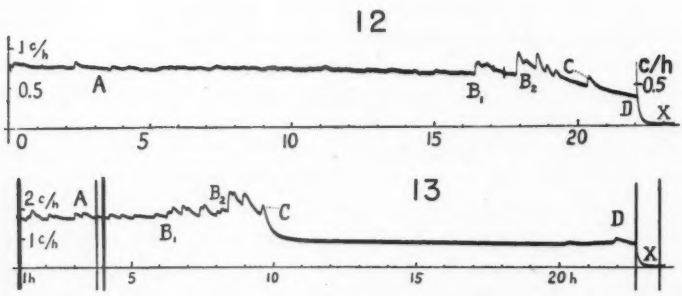
État ou sexe	Poids en grammes	Thermogenèse relative en cal./h. pour 1 gr.		Rapport, M/m	Type
		minimale (m)	maximale (M)		
J 5	0.004	0	10	—	V
J 10	0.01	0	5	—	V
N II	0.01	5	10	2	U
N III	0.013	0	8	—	U
N IV	0.045	3	5	1.6	W
M	0.53	4	6	1.5	R
M	0.87	3	4	1.3	R
M	1.47	1.4	1.8	1.3	R
F	0.55	4	6	1.5	W
F	0.64	3	5	1.6	W
F	0.8	1.7	4	2.3	S
F	1	2	4	2	W
F	1.05	2	5	2.5	S
F	1.08	2	3	1.5	W
F	1.3	3	4	1.3	S

F: femelle; M: mâle; J 5, J 10: jeunes nymphes, 5 ou 10 jours après l'éclosion; N II, N III, N IV: nymphes après la 2e, la 3e, ou la 4e mue.

Enfin, si l'on compare les tableaux I et II, on constate qu'à poids égal la thermogenèse est notablement plus forte à 31.4° qu'à 24.9°: par exemple un individu femelle de *M. differentialis* pesant de 1.3 gr. à 1.5 gr. fournit une thermogenèse maximale de 1.3 cal. h. par gramme à 24.9° et de 4 cal. h. à 31.4°; un individu mâle de 0.8 gr. fournit un maximum de 1.6 cal. h. à 24.9° et 4 cal. h. à 31.4°. Ceci est bien conforme à ce que nous savons de la stimulation du métabolisme par une élévation de température chez les animaux poïkilothermes.

III. Modifications de la thermogenèse de *Melanoplus* en atmosphère confinée

Nous avons recherché quelles modifications subissaient les courbes précédentes lorsqu'on place les animaux en atmosphère confinée. La cellule micro-calorimétrique est cette fois fermée par un bouchon plein, en liège ou en caoutchouc (*c* fig. 2), et ne contient aucun dispositif pour absorber l'anhydride carbonique. Dans ces conditions, l'asphyxie est rapide. La figure 12 a été fournie à 24.9° par un *Melanoplus mexicanus atlanis* femelle, pesant 0.350 gr. Le début de la courbe est très régulier et conforme au type *R* décrit plus haut, avec un débit thermique variant de 1.8 à 2.4 cal.h. par gramme. Au bout de 16 h., en B_1, la courbe change d'allure: l'animal commence à s'agiter en ressentant les premières atteintes de l'asphyxie. En B_2, après 18 h., l'agitation devient plus intense, la thermogenèse prend le type *S* et le débit thermique atteint, en pointe, des valeurs de 3 cal.h. par gramme. A Tian a signalé (13) un semblable crochet de la courbe de thermogenèse



FIGS 12 et 13. Photographies de thermogrammes de *Melanoplus* adultes placés dans des conditions asphyxiques; cellule fermée, sans absorption du gaz carbonique (dispositif *c*, fig. 2), à 24.9°. FIG. 12: *M. mexicanus atlanis* femelle, de 0.350 gr.: d'abord type *R*, puis, en B_1, première réaction à l'asphyxie; en B_2 début du paroxysme de thermogenèse: réalisation du type *S*. En *C D*, décroissance de la thermogenèse; l'animal entre en coma (*D*); *X* retour au zéro, à la fin de l'expérience. FIG. 13: *M. bivittatus* femelle de 0.838 gr.; mêmes phases; *D*: coma prolongé.

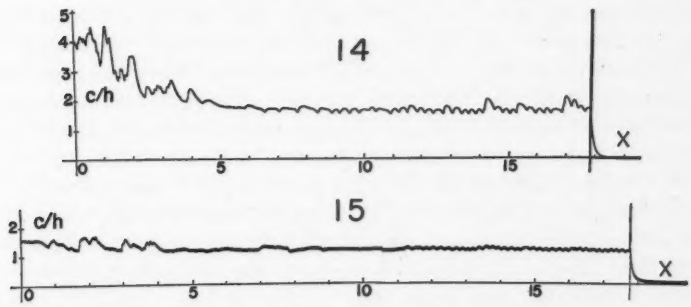
sur des mouches placées en atmosphère confinée, avant la période d'asphyxie. En *C D*, on assiste enfin à une descente de la courbe. A la fin de l'expérience l'insecte est encore vivant, mais plongé dans une sorte de coma; sa thermogenèse n'est plus que 1 cal.h par gramme.

La figure 13 est fournie par un *Melanoplus bivittatus* femelle pesant 0.838 gr. L'asphyxie progresse plus rapidement, l'animal étant plus gros que le précédent, mais on peut distinguer sur la courbe les mêmes phases: au bout de six heures, en B_1, début d'inquiétude; après huit heures, en B_2, début du paroxysme d'agitation (type *S*), enfin après neuf heures et demie, en *C D*, descente de la thermogenèse qui se stabilise à un niveau très bas. Si l'expérience se prolonge, la mort succède insensiblement au coma, sans modification sensible du niveau de la thermogenèse.

Lorsqu'on opère à une température plus élevée, les mêmes symptômes apparaissent au bout d'un temps plus court. Par exemple un *M. differentialis* de 0.8 gr. à 31.4°, montre les premiers signes thermiques d'asphyxie B_1 au bout de quatre heures et le début du paroxysme B_2 au bout de sept heures, au lieu de respectivement six et neuf heures à 24°.

Dans le but de déterminer si ces modifications de la thermogenèse sont dues à l'épuisement de l'oxygène (anoxie) ou bien à l'accumulation du gaz carbonique dans la cellule calorimétrique, nous avons pratiqué d'autres expériences, encore en cellule fermée mais cette fois en présence de soude caustique (dispositif *e* de la figure 2). L'anhydride carbonique se trouve ainsi éliminé au fur et à mesure de sa production. On constate qu'alors l'animal survit pendant un temps beaucoup plus long, sa thermogenèse se stabilisant sur un type particulier.

La figure 14 montre le thermogramme d'un individu mâle de *Melanoplus differentialis* pesant 0.998 gr. et placé à 26.5° en présence de soude. On voit



FIGS 14 et 15. Photographies de thermogrammes de *Melanoplus differentialis* adultes placés en cellule fermée en présence de soude (dispositif *e*, fig. 2), à 26.5°. FIG. 14: mâle pesant 0.998 gr. FIG. 15: femelle pesant 1.096 gr.; passage progressif du type *T* au type *N*.

que, dans les quatre premières heures, la thermogenèse demeure assez accidentée, du type *T*. Puis, à partir de la cinquième heure, elle se stabilise selon un type finement ondulé (*N* fig. 3). L'animal devient alors très calme, son débit thermique prenant une valeur moyenne de 1.6 cal.h. par gramme. Il semble que l'absence totale de gaz carbonique dans l'air respiré, jointe à un abaissement de la tension d'oxygène, "endort" en quelque sorte l'insecte, diminuant son activité musculaire, abaissant sa thermogenèse et lui permettant de survivre très longtemps en ménageant les réserves d'oxygène de la cellule close. Ou peut voir par l'examen du thermogramme 14 que l'animal est encore parfaitement vivant au bout de 18 h. Nous avons prolongé une autre expérience pendant 70 h. et à la fin l'insecte était encore en excellente forme. Or, pour des insectes de ce poids et à cette température, des troubles analogues à ceux des figures 12 et 13 seraient apparus dès la septième heure et la mort au bout de la dixième, si l'on avait opéré en cellule fermée sans la présence d'alcali. Nous devons donc en conclure que les altérations du thermogramme observées plus haut en atmosphère confinée sont dues à l'autointoxication par le gaz carbonique provenant de la respiration et non à l'épuisement des réserves d'oxygène dans le récipient calorimétrique.

La figure 15 présente un thermogramme de *M. differentialis* femelle pesant 1.096 gr., placé à 26.5°, en cellule fermée en présence de soude. On remarquera que la thermogenèse initiale, moins forte que précédemment, tend encore à se déprimer, passant du type *T* au type *R* au bout de quatre heures, puis au type *N* au bout de 11 h. Ici sa valeur d'équilibre n'est plus que de 1.1 cal.h. par gramme et les fluctuations deviennent de très faible amplitude: de l'ordre de 0.1 cal.h., avec une période d'environ 10 m.

La comparaison des trois séries de résultats examinées jusqu'ici (dispositifs *b*, *c* et *e* de la figure 2) nous permet maintenant d'envisager l'hypothèse que l'agitation et les fortes oscillations thermiques obtenues avec le dispositif *b* peuvent être dues en partie à une élimination imparfaite du gaz carbonique

par diffusion à travers les tampons d'ouate. Une partie de l'anhydride carbonique produit par la respiration de l'animal s'accumulerait dans l'atmosphère de la cellule, atteignant en moins d'une heure une valeur sensiblement fixe, comme le démontre l'uniformité du thermogramme d'un bout à l'autre de l'expérience (voir figs 4 à 9). Ce taux serait insuffisant pour provoquer des accidents asphyxiques analogues à ceux des figures 12 et 13; il serait cependant suffisant pour déterminer une stimulation des mouvements de l'insecte, donc de son métabolisme et de sa thermogenèse celle-ci atteignant des maxima plus élevés que lorsqu'on élimine totalement l'anhydride carbonique (figs 14 et 15). Nous nous proposons de serrer de plus près ce problème en modifiant nos dispositifs de façon à pouvoir introduire en cours d'expérience dans la cellule calorimétrique des mélanges gazeux de composition déterminée et à pouvoir suivre à chaque instant les réactions de l'animal en employant l'enregistrement immédiat par photopen.

IV. Thermogrammes de Blattidés

Parmi les Blattidés (*Dictyoptères*) nous avons examiné: *Blattella germanica* (L.), *Blatta orientalis* L., et *Periplaneta americana* (L.). Nous décrirons seulement les thermogrammes de cette dernière espèce, et en premier lieu ceux que nous avons obtenus avec le dispositif *b* de la figure 2, c'est-à-dire en permettant la respiration par diffusion à travers un tampon d'ouate.

Le thermogramme de la figure 16 est fourni par une jeune nymphe de *Periplaneta americana*, au stade qui suit la première mue et d'un poids de 0.041 gr. Il appartient nettement au type *T*, avec toutefois deux séries très différentes de paroxysmes: les uns très marqués, atteignant 0.2 cal.h. en valeur absolue, soit 5 cal.h. par gramme, et espacés d'environ trois heures; les autres peu accentués (0.02 cal.h. au-dessus du minimum: 0.06) et apparaissant avec une période moyenne de 20 m.

Dans le thermogramme 17, fourni par un mâle adulte de la même espèce, pesant 0.787 gr., et pris à 24.9°, on remarque le même type *T* de thermogenèse, mais avec une plus grande régularité; 22 paroxysmes s'échelonnent

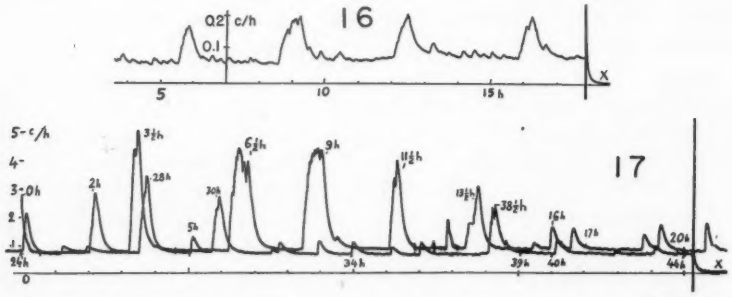


FIG. 16. *Periplaneta americana* nymphe après la première mue; 0.041 gr.; 31.4°; type *T*; respiration par diffusion.

FIG. 17. *Periplaneta americana* mâle adulte; 0.787 gr.; 24.9°; enregistrement sur 44 h.; type *T*; respiration par diffusion.

au long des 44 h. de l'expérience, avec des valeurs décroissantes: 5 cal.h. au début, 1.5 cal.h. à la fin. Ils sont séparés par des phases de repos où la thermogenèse s'abaisse jusqu'à une valeur très constante d'environ 1 cal.h. en se stabilisant plus parfaitement que dans la figure 16. On notera la haute valeur relative des premiers paroxysmes (6.5 cal.h. par gramme), opposée à la faible valeur de la thermogenèse basale (1.1 cal.h. par gramme). Cet écart très accentué entre les valeurs extrêmes de la thermogenèse est caractéristique des *Periplaneta*. On remarquera également sur cette fig. 17, l'existence d'un rythme diurne, en dépit du fait que cette expérience, comme toutes les autres décrites dans ce mémoire, ait eu lieu dans une complète obscurité. Dans la partie gauche du thermogramme, enregistrée durant les après-midi et les soirées, les maxima sont en effet plus accentués que dans la partie droite, enregistrée entre deux heures du matin et midi.

La figure 18 est la photographie d'un enregistrement obtenu au moyen de la "Photopen" Beckman, que nous avons mentionnée plus haut. Contrairement aux précédents, ce graphique doit être lu de droite à gauche. On remarquera sa parfaite similitude avec celui de la figure 17, à part de très petites différences dans les détails du tracé, montrant par là que la forme du thermogramme est bien indépendante du procédé d'enregistrement. Il s'agit dans les deux expériences d'un mâle adulte de *Periplaneta americana*. Dans la seconde (fig. 18) la thermogenèse basale tombe à 0.7 cal. h. par gramme, alors que les maxima peuvent encore atteindre 5 cal. h. Chez cet animal, l'écart de thermogenèse entre l'état de repos et l'état d'activité peut donc être de 1 à 7.

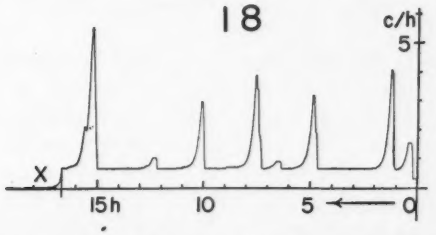


FIG. 18. Enregistrement par "Photopen" du thermogramme d'un mâle adulte de *Periplaneta americana*, pesant 1.004 gr.; à 26.5°; respiration par diffusion. Contrairement aux précédents, ce graphique doit se lire de droite à gauche.

Nous avons rassemblé dans le tableau III les résultats obtenus sur divers individus de *Periplaneta americana*, en outre de ceux qui ont été fournis dans les figures 16 à 18. Nous pouvons en tirer les conclusions suivantes:

1. Il existe de grandes variations individuelles dans la valeur de la thermogenèse, principalement chez les formes larvaires.

2. La thermogenèse relative minimale est, dans l'ensemble, plus élevée chez les nymphes que chez les adultes, atteignant de 0.8 à 3.5 cal. h. par gramme chez les premières, et de 0.8 à 1.4 cal. h. chez les seconds.

TABLEAU III

THERMOGENÈSE DE *Periplaneta americana* L.

État ou sexe	Poids en grammes	Tempér. degrés centigr.	Thermogenèse relative en cal. h. pour 1 gr.		Rapport M/m	Type
			minimale (m)	maximale (M)		
N I	0.041	31.4	2	5	2.5	T
N I	0.042	31.4	0.8	2.5	3	W
N II	0.137	31.4	1.8	4.5	2.5	T W
N II	0.142	31.4	2.8	3.5	1.25	W
N IV	0.200	31.4	1.5	2	1.3	S W
N IV	0.209	31.4	3.5	4.5	1.3	W
N V	0.408	24.9	0.8	1	1.2	W
N VI	0.540	31.4	1.8	4.5	2.5	S
M	0.731	24.9	0.8	4	5	S N
M	0.787	24.9	1	6	6	T
M	0.821	24.9	1.2	3.6	3	T
M	1.004	26.5	0.6	5	8.3	S
M	1.094	24.9	1.3	4.5	3.5	S T
F	0.867	26.5	1.4	1.7	1.2	T
F	0.984	26.5	0.8	1.8	2.2	T
F	1.478	24.9	1	3.4	3.4	S

F: femelle; M: mâle; N I, N II, N IV, N V, N VI: nymphes après la première, 2e, 4e, 5e ou 6e mue.

3. La thermogenèse maximale est plus élevée chez les mâles que chez les femelles, atteignant de 3.6 à 6 cal. h. par gramme chez les premières et de 1.7 à 3.4 chez les secondes. Chez les larves, elle est très variable, oscillant entre 1 et 5 cal. h.

4. Le rapport de la thermogenèse maximale à la thermogenèse minimale, M/m, est très élevé chez les mâles adultes, atteignant de 3 à 6 et même 8. Chez les femelles et les larves, il reste plus faible, variant entre 1.2 et 3.4.

5. Le mode dominant de thermogenèse chez les nymphes et du type irrégulier *W*; puis, avec le vieillissement de l'animal, il passe au type *S* ou *T*.

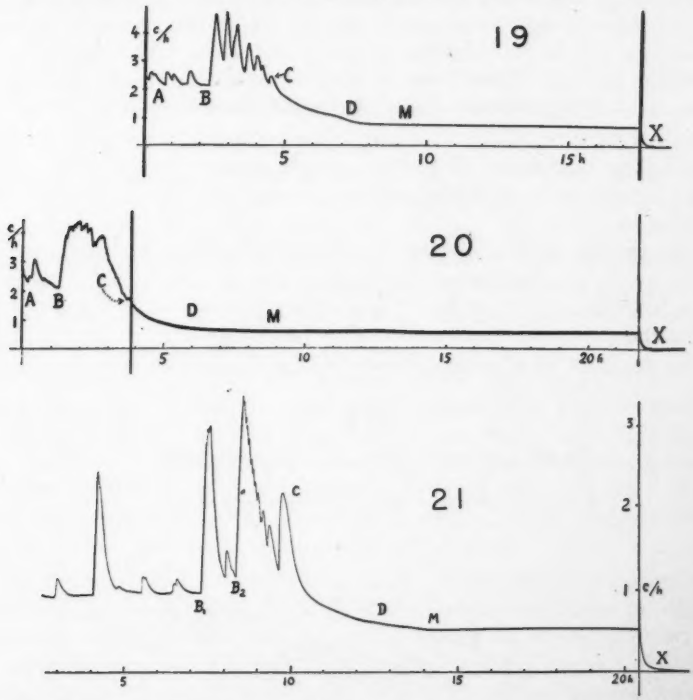
6. Depuis les premiers stades larvaires jusqu'à l'adulte, la thermogenèse relative varie peu en fonction de la masse de l'animal. Une très jeune nymphe pesant quatre centigrammes présente par exemple des thermogenèses extrêmes de 0.8 et 2.5 cal. h. par gramme, alors qu'une femelle adulte pesant 1.478 gr., soit 37 fois plus, fournit 1 et 3.4 cal. h. par gramme et une autre femelle, pesant 0.984 gr., donne 0.8 et 1.8 cal. h. par gramme, différences faibles si l'on tient compte de l'écart considérable des masses.

Si nous comparons ces résultats à ceux que nous avons obtenus avec les *Melanoplus* et mentionnés plus haut, nous voyons que les thermogrammes présentent certaines analogies dans les deux cas; on peut en effet les ranger dans les mêmes types généraux: *S*, *T*, *W*. Par contre on doit relever que, chez *Periplaneta*, l'écart est plus grand que chez *Melanoplus* entre la thermogenèse maximale et la thermogenèse minimale. La comparaison des tableaux I, II et III montre en effet que le rapport M/m atteint, chez le mâle adulte,

des valeurs de 3 à 8.3 chez *Periplaneta* et de 1.3 à 2.5 chez *Melanoplus*. Chez les femelles et les larves, la différence est moins frappante: 1.2 à 3.4 chez *Periplaneta* contre 1.3 à 2.5 chez *Melanoplus*. Ceci prouve que, chez les mâles de *Periplaneta*, l'effort musculaire peut entraîner des paroxysmes de débit thermique très élevés et soutenus pendant une demi-heure ou une heure, tandis qu'au repos la thermogenèse basale peut retomber à un niveau très faible; cette sorte d'"élasticité thermique" est peut-être en relation avec la remarquable vitalité de ces insectes et avec leur résistance bien connue, qui en font un des fléaux des habitations.

V. Thermogenèse de Periplaneta en Atmosphère Confinée

Les figures 19, 20, 21 ont été obtenues avec des *Periplaneta americana* placées dans les conditions de la figure 2*c*, c'est-à-dire en cellule fermée sans alcali. On voit que les blattes traitées de cette façon manifestent les mêmes altérations de la thermogenèse que les *Melanoplus* examinés plus



FIGS 19 et 20. Thermogrammes d'individus adultes de *Periplaneta americana* placés en cellules closes, sans absorption du gaz carbonique (*c*, fig. 2), à 24.9°: FIG. 19: mâle de 0.76 gr.; en *A*, type *S*; en *B C*, paroxysme asphyxique; en *M*: mort; *X*: retour au zéro. FIG. 20: femelle de 1.29 gr.; type *T*.

FIG. 21. Nymphe de *Periplaneta americana* après la 6e mue, pesant 0.54 gr., placée en cellule close sans alcali à 31.4°. D'abord type *S*; en B_1, premiers symptômes asphyxiques; en B_2 *C*, paroxysme; *D*: coma; *M*: mort; *X*: retour au zéro.

haut. En *B*, les premiers symptômes de l'asphyxie se manifestent par une montée rapide de la thermogenèse. Cette montée survient au bout de 1½ h. dans le thermogramme 20, fourni par une femelle adulte pesant 1.29 gr.; au bout de 2½ h. dans le thermogramme 19, donné par un mâle adulte de 0.76 gr.; au bout de 7½ h. dans le thermogramme 21, qui est celui d'une nymphe prise après la sixième mue et pesant 0.54 gr. On voit que ces durées sont en proportion inverse du poids des animaux, ce qui était à prévoir. En ce qui concerne les types de thermogenèse, les figures 19 et 21 appartiennent au type *S*, la figure 20 au type *T*. La mort survient en *M*, respectivement au bout de 8, 7, et 14 h.

Comme dans le cas des *Melanoplus*, nous avons cherché à reconnaître si les altérations observées dans la thermogenèse étaient dues à l'accumulation d'anhydride carbonique ou à l'épuisement des réserves d'oxygène de la cellule calorimétrique. Nous avons pour cela utilisé le dispositif (*e*) de la figure 2, plaçant un peu de soude caustique dans le fond de la cellule. La figure 22 montre un thermogramme obtenu dans ces conditions avec une *Periplaneta americana* femelle adulte pesant 0.867 gr. L'animal conserve sa pleine vitalité jusqu'à la fin de l'expérience, qui a duré 18 h. Au début le thermogramme est du type *T*; au bout de 12 h. il se modifie: les paroxysmes sont plus espacés et séparés par des périodes de repos légèrement ondulées se rapprochant du type *N* que nous avons décrit chez les *Melanoplus* placés dans des conditions analogues. Il semble, que là encore, l'élimination complète du gaz carbonique et de la stimulation qu'il procure, place l'animal dans un état de repos.

Sur un certain nombre de thermogrammes nous avons enregistré, même après la mort, un dégagement de chaleur appréciable, ainsi qu'on peut le voir sur les figures 19, 20 et 21. Nous nous proposons de rechercher la cause de cette thermogenèse "post mortem". Jusqu'ici nous ne l'avons observée que chez des insectes et seulement dans le cas de mort en état d'asphyxie.

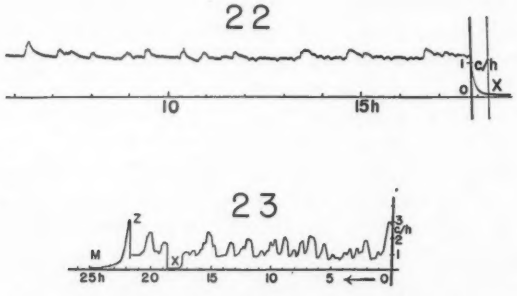


FIG. 22. Thermogramme d'une femelle adulte de *Periplaneta americana*, pesant 0.867 gr., placée en cellule fermée en présence de soude (dispositif *e*, fig. 2) à 26.5°. D'abord type *T*, tendant progressivement vers le type mixte *N T*.

FIG. 23. Enregistrement par "Photopen" du thermogramme d'une femelle adulte de *Periplaneta americana* pesant 1.142 gr., à 26.5°, dans les conditions du dispositif *a* de la figure 2. En *Z* on fait tomber sur l'insecte 1 cc. d'alcool à 90%. En *M*: mort. En *X*: vérification du zéro. Ce graphique doit se lire de droite à gauche.

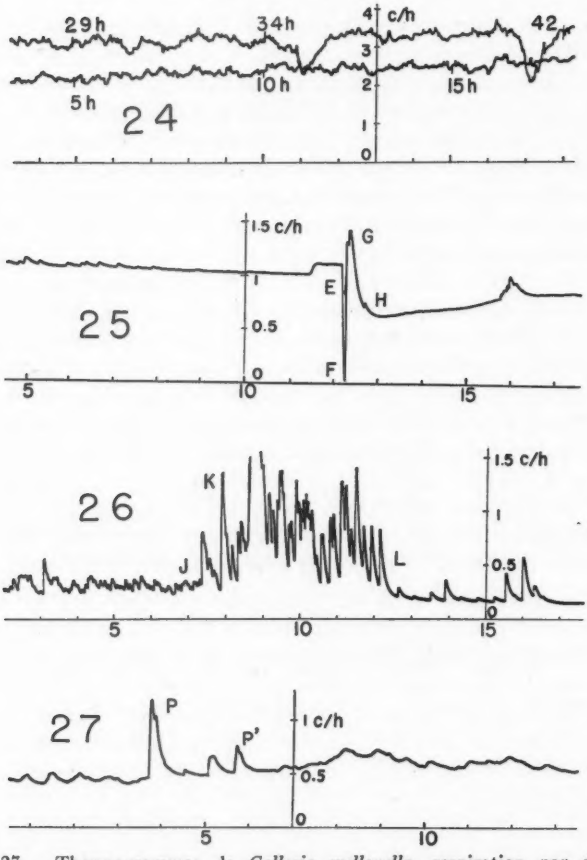
La figure 23 montre l'enregistrement à la "photopen" du thermogramme d'une femelle adulte de *Periplaneta americana* pesant 1.142 gr. à 26.5° et placée dans les conditions du dispositif *a* de la figure 2, avec un centimètre cube d'alcool à 90% dans le tube *W*. La première partie du tracé, qui doit se lire de droite à gauche, est parfaitement normale et de type *T*, fournissant dans les paroxysmes 2.3 cal. h. et dans les minima 0.7 cal. h., soit un rapport M/m égal à 3, valeur fréquente chez les femelles. Ces chiffres représentent des thermogenèses relatives extrêmes de 2 et 0.6 cal. h. par gramme. Au bout de 17 h., en *X*, nous avons fait un retour au zéro pendant une heure, pour contrôle. Puis, au bout de 21 h. 45 m., en agissant sur la poire *K* (voir *a*, fig. 2), nous avons laissé tomber l'alcool sur l'insecte. On constate, en *Z*, l'élévation rapide de la thermogenèse due à l'agitation de l'animal, en réponse à l'excitation. Le débit thermique atteint alors en pointe 3 cal. h., soit une valeur relative de 2.6 cal. h. et le thermogramme affecte, pour ce paroxysme, le type *S*. Ensuite, la thermogenèse décroit rapidement et deux heures plus tard, l'animal meurt. Dans ce cas, on n'observe pas de thermogenèse post mortem.

VI. Variations de la thermogenèse en fonction des stades de développement chez quelques Lépidoptères

Dans les exemples précédents nous avons pu constater que la forme du thermogramme se modifiait sensiblement en fonction du stade de développement. Toutefois il ne s'agissait jusqu'ici que d'insectes à métamorphoses incomplètes; la thermogenèse conservait donc chez eux de notables ressemblances d'un stade à l'autre. Nous avons poursuivi d'autres séries d'expériences, cette fois sur des insectes holométaboles, et observé, comme on pouvait le prévoir, des différences plus étendues entre les thermogrammes des larves et ceux des adultes.

Bell, Taylor et Crescitelli (1, 11) ont signalé les variations que subit l'énergie calorifique dégagée au cours de la pupaison de *Galleria mellonella* (L.). A poids égal cette énergie est d'abord plus élevée chez le mâle, puis chez la femelle; au total la femelle l'emporte. La chaleur dégagée augmente lorsque la température ambiante s'élève: à 25° C. Bell a trouvé chez le mâle un dégagement total de 625 cal. par gramme; chez la femelle 698; à 40° C. les chiffres deviennent respectivement 960 et 1057 (1, 10).

Appliquant à *Galleria mellonella* la méthode d'analyse micro-calorimétrique décrite au sujet des *Melanoplus* et des *Periplaneta*, nous avons pu détailler les modalités de la thermogenèse à tous les stades du développement. Le thermogramme 24 a été fourni, à 24.9°, pendant 42 h., par une chenille de *Galleria mellonella* parvenue à son stade final (IX). Il est du type irrégulier (*W*). Quand la chenille a filé un cocon et s'est transformée en pupe, sa thermogenèse diminue et devient du type *R* (fig. 25, dans la partie gauche). A la fin de la pupaison l'éclosion du papillon, crise majeure dans l'existence de l'insecte, s'inscrit sur la courbe de thermogenèse sous forme d'une descente brusque *E F* (fig. 25), suivie d'une remontée également brusque, *F G*, et



FIGS 24 – 27. Thermogrammes de *Galleria mellonella*, respiration par diffusion: FIG. 24. Chenille au dernier stade (IX), peu avant la préparation du cocon, à 24.9°; type *W*. Le double tracé provient de ce que l'expérience a duré 43 h.

FIG. 25. Eclosion du papillon. Chrysalide pesant 0.190 gr.; à 31.4°. D'abord la thermogenèse de la pupe est du type *R*. Puis, au moment de l'éclosion, chute de la thermogenèse en *E F*, suivie d'une remontée brusque *F G* (type *V*). Ensuite baisse progressive en *G H*. Le papillon nouvellement éclos présente d'abord une thermogenèse basse (*H*); puis elle remonte progressivement en prenant le type *R T*.

FIG. 26. Papillon mâle, 0.06 gr.; 24.9°, type *W S*. Paroxysme de thermogenèse (agitation) en *J K L*; puis type *S*, plus régulier.

FIG. 27. Papillon femelle; 0.069 gr.; 31.4°. En *P P'*, ponte, se manifestant par des paroxysmes, de type *S*, de la thermogenèse; 14 œufs sont pondus au total.

d'une descente plus progressive, *G H*. Le papillon nouvellement éclos présente d'abord une thermogenèse assez faible, inférieure à celle de la pupe, avec quelques paroxysmes espacés (type *R T*); puis sa thermogenèse s'affirme (*J K*, fig. 26) tout en prenant le type *S*. Elle atteint un paroxysme de 1.5 cal. h., pour un mâle de 0.060 gr., lorsque l'animal bat des ailes (*K L*). Enfin, lorsqu'il est épuisé (*L*), elle retombe à une valeur très faible (0.2 cal. h.), tendant alors vers le type *R S*.

Dans la vie de l'insecte, un autre événement important s'inscrivant dans les thermogrammes est la ponte. Le tracé 27 a été fourni par un papillon femelle pesant 0.069 gr. à 31.4°. En *P P'* l'insecte effectue sa ponte, effort se traduisant par de brusques augmentations de la thermogenèse, qui s'accroît passagèrement de 150%, passant de 0.5 à 1.25 cal. h. Ensuite la thermogenèse retombe à un régime plus modeste, de type *W*, variant de 0.5 à 0.6 cal. h. Chez la femelle la thermogenèse basale relative demeure plus élevée que chez le mâle; en compensation les paroxysmes atteignent des valeurs de pointe moins élevées.

Ces exemples confirment notre remarque: tous les événements survenant au cours de la vie d'un animal: vieillissement organique, métamorphoses, phénomènes de la reproduction, nutrition, réactions à des facteurs physiques ou chimiques, s'inscrivent dans le tracé des thermogrammes et cette inscription nous fournit un excellent moyen d'évaluer leur influence sur le métabolisme et leurs modalités.

Nous avons réuni sur la tableau IV quelques données fournies par *Galleria mellonella* à divers stades de développement. Les chenilles fournissent des thermogrammes de type *W* avec peu d'écart entre les maxima et les minima (M/m = 1.2). Cependant, juste avant la pupaison, cet écart s'amplifie. La pupe fournit moins de chaleur que la chenille: un minimum de 3 cal. h.

TABLEAU IV

THERMOGENÈSE DE *Galleria mellonella* (L.)

Stade ou sexe	Poids en grammes	Tempér. degrés centigr.	Thermogenèse relative en cal. h. pour 1 gr.		Rapport, M/m	Type
			minimale (m)	maximale (M)		
L IX	0.196	24.9	11	13	1.2	W
L IX B	0.196	24.9	10	17	1.7	W
P M	0.095	24.9	3	5	1.7	R
P M	0.095	31.4	5	7	1.4	R
P F	0.129	24.9	3	4.5	1.5	R
P F	0.120	31.4	8	9	1.1	R
E M	0.094	24.9	0.5	7	14	V
E M	0.095	31.4	0.1	10	100	V
E F	0.129	24.9	2.3	11	5	V
E F	0.120	31.4	0.08	11	130	V
M	0.060	24.9	3	25	8	S
M	0.074	24.9	2.7	11	4	S
M	0.075	31.4	6	12	2	R S
M	0.032	31.4	8	12	1.5	R S
F	0.104	24.9	4	6	1.5	S
F	0.099	24.9	2	4	2	S
F	0.069	31.4	7	10	1.4	W
F P	0.120	31.4	4	17	4	S
F P	0.069	31.4	7	18	2.5	S

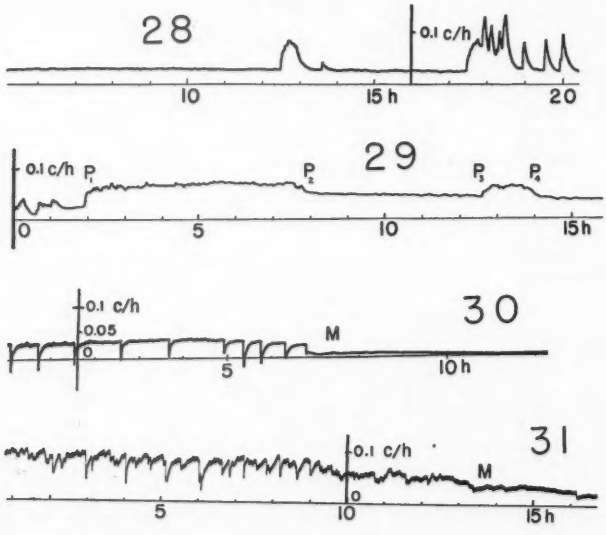
L IX: chenille au stade IX; L IX B: chenille juste avant la pupaison; P M: pupe mâle; P F: pupe femelle; E M: éclosion de l'imago mâle; E F: éclosion de l'imago femelle; M: papillon mâle; F: papillon femelle; F P: papillon femelle pendant la ponte.

par gramme à 24.9° au lieu de 10 à 11 chez la chenille. Le rapport entre maximum et minimum demeure faible chez la pupe: de 1.1 à 1.7; sa thermogenèse est du type quiescent *R*.

L'éclosion du papillon se manifeste, comme nous l'avons vu, par une chute brusque de la thermogenèse (type *V*), entraînant une élévation passagère considérable du rapport M/m. Celui-ci peut atteindre des valeurs de 100 à 130, surtout à 31.4°, température voisine de l'optimum pour le développement de *Galleria*. Nous avons observé en effet des minima plus bas à cette température qu'à 24.9°, les maxima demeurant peu changés.

En ce qui concerne les adultes, la thermogenèse prend d'abord le type *S* et sa valeur basale demeure du même ordre de grandeur dans les deux sexes: 2 à 4 cal. h. par gramme à 24.9°; 4 à 8 cal. h. à 31.4°. Mais le papillon mâle peut fournir des maxima plus élevés que la femelle, surtout avant l'accouplement; à ce moment le maximum de thermogenèse peut atteindre, pour le mâle, 25 cal. h. par gramme à 24.9°, et le rapport M/m s'élever jusqu'à 8. Pour la femelle les maxima sont observés au moment de la ponte, atteignant 18 cal. h. par gramme. Après l'accouplement le thermogramme du mâle tend vers le type *R S* et, après la ponte, celui de la femelle vers le type *W*.

Nous avons analysé la thermogenèse de divers autres Lépidoptères: *Malacosoma*, *Diarsia*, *Tineola*, observant des faits comparables aux précédents.



FIGS 28–31. Thermogrammes de *Tineola* et de *Drosophila* à 24.9°: FIG. 28. *Tineola bisselliella:* papillon mâle, 0.003 gr.; tube fermé; type *R T S*.
FIG. 29. *Tineola bisselliella:* papillon femelle, 0.003 gr., tube fermé; type *R*. En P_1 P_2 et P_3 P_4 périodes de ponte (six œufs pondus au total).
FIG. 30. *Drosophila melanogaster*: un seul individu femelle, poids 0.001 gr.; type *V*.
FIG. 31. *Drosophila melanogaster*: cinq individus femelles; type *V*. En *M*, mort de la plupart des animaux: un seul survit à la fin de l'expérience, après 17 h.

Nous présenterons ici seulement deux thermogrammes de *Tineola bisselliella* (Hum.), la tisseuse (teigne) des vêtements, qui offrent un intérêt spécial en raison de la très petite taille des animaux. Ceux-ci pèsent de un à trois milligrammes et les quantités de chaleur dégagées sont de quelques dix millièmes de calorie par minute. La sensibilité de l'appareil est cependant suffisante pour enregistrer clairement ces faibles dégagements de chaleur, ainsi qu'on peut le voir sur les figures 28 et 29.

Le thermogramme 28 est fourni par un mâle de *T. bisselliella* pesant 1.5 mg. D'abord très calme et du type *R* il fournit un paroxysme isolé de type *T* au bout de 12 h.; puis, au bout de 17 h., il entre dans une phase d'agitation, du type *S*, avec un rapport M/m s'élevant jusqu'à 6. Les valeurs absolues du débit thermique sont faibles: 0.02 cal. h. au minimum et 0.13 au maximum; mais cela nous donne des valeurs relatives de 13 et de 87 cal. h. par gramme d'animal. Ces chiffres sont beaucoup plus élevés que tout ce que nous avons rencontré jusqu'ici chez *Melanoplus*, *Periplaneta*, et *Galleria*. Toutefois cela n'est pas pour nous surprendre, cette thermogenèse élevée de *Tineola* étant bien conforme à ce que nous savons du métabolisme très actif des petits animaux.

La thermogramme 29 est celui d'une femelle de *T. bisselliella*, pesant trois milligrammes et en train d'effectuer sa ponte. On peut y remarquer deux niveaux extrêmes du débit thermique: l'un représenté en P_1 P_2 et P_2 P_3, d'une valeur d'environ 0.07 cal. h., soit 20 cal. h. par gramme, l'autre, offert au début de la courbe, de 0.025 cal. h., soit 8 cal. h. par gramme. La première valeur semble correspondre aux efforts de l'animal au moment de la ponte. Entre P_2 et P_3, un niveau intermédiaire, de 0.04 cal. h., correspond à une phase de repos. Le rapport M/m entre les extrêmes est de 2.5; il est moins élevé que chez le mâle, comme nous l'avions vu aussi chez *Galleria*.

VII. Thermogrammes de Drosophiles

Nous avons enregistré les thermogrammes de plusieurs Diptères, des genres *Cynomyia*, *Musca*, *Tipula*, et *Drosophila*. Nous ne présenterons ici que ceux de ce dernier genre, spécialement intéressant en raison de sa faible taille.

La figure 30 est la photographie d'un thermogramme obtenu avec un seul individu femelle de *Drosophila melanogaster* Meig., pesant 1.2 mg. Le tracé est de type *V*. La thermogenèse atteint ici, à son maximum, 0.03 cal. h., quantité faible en valeur absolue mais très forte en valeur relative, puisqu'elle représente 25 cal. h. par gramme. Ce chiffre élevé, comparable à ceux que nous avons signalés chez les *Tineola*, est d'un ordre de grandeur fréquent chez les très petits insectes, le faible volume de l'animal signifiant proportionnellement une grande surface de rayonnement et un fort métabolisme basal.

La forme du thermogramme 30 présente aussi un certain intérêt: Elle est du type *V*, que nous avons rencontré déjà à plusieurs reprises chez les formes larvaires de *Melanoplus*, et, d'une façon générale, chez les individus de très

petite taille. Ce mode de thermogenèse est caractérisé par des paliers légèrement ondulés et relativement élevés, coupés par des chutes brusques, de très courte durée. Ces dernières sont si accentuées que la thermogenèse peut s'y annuler complètement et même être remplacée par une absorption de chaleur. Pour expliquer celle-ci on peut émettre l'hypothèse de l'évaporation passagère d'un liquide. En effet nous n'avons observé de telles manifestations endothermiques que chez de très petits animaux, d'une taille insuffisante pour saturer d'humidité l'atmosphère de la cellule calorimétrique. Chez eux une certaine évaporation demeurait donc possible, ce qui n'était pas le cas pour des animaux plus volumineux.

Dans l'expérience 30 les chutes de thermogenèse sont d'abord espacées de une heure à une heure et demie; puis elles deviennent plus fréquentes, survenant toutes les 25 ou 40 m.; en même temps les paroxysmes s'abaissent de 0.03 à 0.02 cal. h., jusqu'à la mort, qui survient ici sept heures après le début de l'expérience (*M*, fig. 30).

La petite taille de la Drosophile, si elle constitue une difficulté pour mesurer la thermogenèse d'un individu isolé, va nous ouvrir, par contre, d'intéressantes possibilités pour l'étude du comportement de groupes d'animaux. La figure 31 présente un thermogramme obtenu avec cinq individus femelles de *D. melanogaster*, pesant au total 6 mg., à 24.9°. On notera que les maxima sont relativement moins élevés que dans le cas précédent: 0.1 cal. h. au total soit 17 cal. h. par gramme. D'autre part les minima n'atteignent jamais de valeurs nulles ou négatives. Cependant il est remarquable qu'ils soient encore aussi accentués, ce qui prouve que les animaux synchronisent dans une certaine mesure leur activité: entrant en repos simultanément puis reprenant collectivement leur agitation. Cela conserve dans une certaine mesure au thermogramme le caractère *V*, tout en introduisant l'irrégularité due à la présence des cinq individus indépendants. Au total le type du thermogramme peut donc être décrit comme *V W*. A partir du point *M*, quatre des Drosophiles étant mortes, le tracé devient celui d'un individu unique, d'ailleurs épuisé et de type *R*.

VIII. Thermogrammes de *Cicindela*

Parmi les Coléoptères nous avons enregistré des thermogrammes de *Carabus*, *Anthrenus*, *Phyllophaga*, et *Cicindela*. La figure 32 présente le thermogramme d'une *Cicindela sexguttata*, Fabr., pesant 0.073 gr., à 31.4°. On notera son caractère particulier, consistant en des paroxysmes de type *S*, séparés par des paliers de type *N*. Au total le thermogramme réalise un type

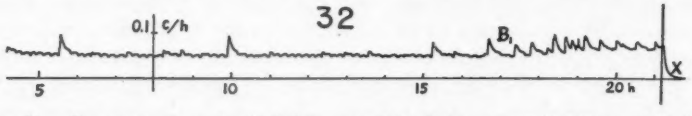


FIG. 32. Thermogramme de *Cicindela sexguttata* Fabr. pesant 0.073 gr., à 31.4°, en tube fermé. Type *S N*. En B_1, l'animal ressent les premiers effets de l'asphyxie; la thermogenèse prend le type *S W*. A la fin de l'expérience, toutefois, au bout de 21 h., l'insecte est encore vivant. *X* retour au zéro.

composite *S N*, que nous n'avons pas rencontré jusqu'ici. Comme l'expérience 32 est réalisée en tube fermé, sans soude, on peut noter en B_1, au bout de 16 h., une modification du thermogramme, montrant les premières réactions de l'animal à un début d'asphyxie. Toutefois celle-ci n'en est encore qu'à ses débuts et, à la fin de l'expérience, au bout de 21 h., l'insecte est encore bien vivant. Les thermogenèses relatives extrêmes sont de 7 et 14 cal. h. par gramme et leur rapport M/m est donc égal à 2.

Conclusions

Nous avons décrit les modalités de la thermogenèse de quelques insectes appartenant aux ordres des Orthoptères, des Dictyoptères, des Lépidoptères, des Diptères, et des Coléoptères: cinq espèces de *Melanoplus*; des *Periplaneta*, *Galleria*, *Tineola*, *Drosophila* et *Cicindela*. Les thermogrammes ont été obtenus au moyen d'un micro-calorimètre Calvet, soit par enregistrement photographique soit par inscription immédiate grâce à une "Photo-pen".

1. Nous avons distingué parmi eux plusieurs types de régimes, que nous avons désignés par les lettres: *R*, *S*, *T*, *U*, *V*, *W*, *N*. Ils sont fonction du stade du développement, de l'âge, de l'activité musculaire, des conditions de l'ambiance: température, échanges respiratoires, etc. Ils présentent des différences spécifiques, sexuelles et individuelles. Nous les avons reconnus en premier lieu chez *Melanoplus* et retrouvés dans les autres genres étudiés.

2. Nous avons analysé les modifications du thermogramme lorsque l'insecte est placé dans une cellule close. Le débit thermique s'élève quand l'animal commence à ressentir les premiers effets de l'asphyxie; puis, la thermogenèse passe par un paroxysme saccadé; enfin elle décline rapidement et se stabilise à un niveau très bas. L'insecte se trouve alors dans une sorte de coma, qui passe insensiblement à la mort sans discontinuté de la courbe.

3. Nous avons recherché si ces phénomènes asphyxiques étaient dus à l'accumulation de gaz carbonique ou à l'épuisement des réserves d'oxygène de la cellule calorimétrique. Renouvelant ces expériences en présence de soude caustique, nous avons constaté qu'alors la survie était beaucoup plus longue; le thermogramme passe simplement à un type uniforme et finement ondulé *N*. Donc les modifications de la thermogenèse signalées plus haut sont imputables à l'autointoxication par l'anhydride carbonique dégagé par l'animal, et non au défaut d'oxygène.

4. Nous avons analysé les variations de thermogenèse liées au stade de développement, comparant les divers états larvaires aux adultes, en particulier chez *Melanoplus*, *Periplaneta*, et *Galleria*. Nous avons observé de fortes altérations du thermogramme au moment des mues, de l'éclosion de l'imago et de la ponte. Les larves molles, telles les chenilles, ont une thermogenèse de type *W*.

5. Chez *Periplaneta americana* les maxima de thermogenèse sont relativement plus élevés chez le mâle que chez la femelle. Il en est de même pour le rapport M/m entre les valeurs extrêmes du débit thermique. Entre 25° et 26° C. ce rapport varie de 3 à 8 chez le mâle, de 1.2 à 3.4 chez la femelle.

Chez le mâle les paroxysmes sont abrupts, du type *S*; chez la femelle ils sont plus atténués, du type *T*. Chez les *Melanoplus* on n'observe pas une semblable disparité du rapport M/m et des valeurs relatives de la thermogenèse entre le mâle et la femelle.

6. Parmi les genres étudiés, *Periplaneta*, *Tineola* et *Galleria* sont remarquables pour les fortes valeurs du rapport M/m, qui, chez les mâles, peut atteindre 8, c'est-à-dire beaucoup plus que tout ce que l'on observe chez les *Melanoplus* ou *Cicindela* (2.5 au maximum).

7. Si nous comparons les valeurs relatives de la thermogenèse maximum dans les divers genres étudiés, entre 24 et 32° C., nous trouvons, pour un gramme d'animal adulte, en calories-heures: *Melanoplus*: 1.4 à 3; *Periplaneta*: 1.7 à 6; *Galleria*: 4 à 25; *Cicindela*: 14; *Drosophila*: 10 à 30; *Tineola*: 8 à 80. Dans l'ensemble les animaux les plus petits offrent la thermogenèse relative la plus élevée. Cependant cette règle n'est pas absolument générale: nous avons constaté que tel gros individu de *Melanoplus* peut parfaitement présenter une thermogenèse relative (c'est-à-dire pour un gramme) plus forte qu'un autre individu pesant deux fois moins.

Ces quelques points ne sont que le début d'une série d'observations plus étendues. Nous nous proposons d'enregistrer les thermogrammes de nombreuses autres espèces d'insectes, choisies dans tous les ordres; ceci aux différents stades de développement, à diverses températures, et dans différentes conditions d'échanges gazeux. Après avoir analysé et comparé leurs thermogrammes nous pourrons en tirer des conclusions d'ensemble sur la thermogenèse des insectes et établir un parallèle avec celle des autres invertébrés et des vertébrés que nous avons examinés par ailleurs. Mais, dès maintenant, les résultats obtenus nous semblent suffisants pour montrer quel moyen d'étude précieux représente la micro-calorimétrie, au service des recherches de physiologie comparée, chez les insectes comme chez tous les autres groupes d'êtres vivants.

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REPRODUCTION AND GROWTH IN THE CREEPING VOLE, *MICROTUS OREGONI SERPENS* MERRIAM[1]

By Ian McT. Cowan and Margaret G. Arsenault

Abstract

The growth and reproduction of the cricetine rodent, *Microtus oregoni serpens* Merriam, has been studied in the laboratory and in the wild. Growth data arise largely from 28 captive born litters. It has been determined that gestation is 23½ to 25 days; that mean litter size is 2.95; that a postparturient oestrus is usual but frequently does not occur; that puberty occurs at 22–24 days in females and 34–38 days in males; that there is a sterile period of 5 to 14 days between first oestrus and first conception; longevity in captivity exceeded 320 days but in the wild there was a complete annual turnover. Instantaneous relative growth rates have been determined for four distinguishable phases of growth. A limited experiment using light and heat to stimulate increased reproduction had equivocal results.

Reproduction in the abundant and widely distributed genus *Microtus* has been the subject of several studies. In the main these have dealt with *Microtus agrestis* (2, 3, 7); *M. pennsylvanicus* (4); and *M. californicus* (5), all of them terrestrial species living considerable parts of their lives on the surface of the ground in grassland. The subject of the present study is probably one of the most fossorial species of the genus, approaching *Pitymys* in general habits. Accordingly it was felt that it was probably under quite different environmental pressures than the less specialized species and might consequently reveal important differences from that species in its reproductive biology.

Microtus oregoni serpens Merriam is included in the subgenus *Chilotus* consisting of small semisubterranean voles with dense plushlike fur in which the guard hairs are suppressed; small ears; short tail; and five plantar tubercles.

It is a forest edge species invading cultivated land adjacent to wooded areas, and it sometimes becomes a serious agricultural pest. It constructs long and devious subterranean burrows of its own that, where litter exists, are joined by narrow surface runways. It characteristically makes use of the burrows of the Coast mole, *Scapanus orarius*, for its own purposes. Its distribution is confined to the Puget Sound Lowlands Biotic Area (10) of extreme south coastal British Columbia and adjacent parts of the State of Washington. This area has a mesothermal, humid climate with rainfall at all seasons, mean summer temperature of 63° F., and mean winter temperature of 38° F.

The present study is based upon a laboratory colony established to provide more intimate details of reproduction. The data from this source were supplemented by field studies during which 107 animals were dissected. These animals were taken between March, 1948, and March, 1949. Twenty-eight litters were born to the captive animals before a brief failure of the water supply killed almost the entire stock.

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Contribution from the Department of Zoology, University of British Columbia, Vancouver, British Columbia.

From March to mid-September the colony was maintained at outside temperatures; through the winter however a temperature varying from 57 to 68 degrees was provided.

An activity cage such as described by Morris (9) was constructed. It was found most useful in studying activity patterns and periods. The graphs provided by this cage were found to give a very accurate record of the time and duration of birth of litters.

The field work and the maintenance of the colony were undertaken largely by the junior author.

Early Development

Weight at birth and growth rate of young were recorded on 14 litters totalling 41 young. All were born during the period April to June. Times given for the several events are the modes and, as the litters were examined at 24 hr. intervals, it is to be understood that the observed timing of events is within this latitude.

At birth the young are completely naked, pink, with eyes and ears closed and the pinnae folded forwards. At the end of 12 hr. downlike whiskers have appeared, and the dorsum is turning gray. At the end of 24 hr. the back is blackish and the hair tips have appeared as a soft fuzz. By two and one-half days the dark pigment has spread to the upper surfaces of the legs and tail, and at the same time, the pinnae begin to unfold. At three and one-half days the color of the dorsal hair is evident and the incisors are visible but not yet erupted. The first sparse ventral fur appears at three and one-half days, two days later pigment has formed on the pectoral area, and by eight days it has spread backwards and the animals are almost completely furred. The incisors have erupted by five and one-half days, the lower ones first; muscular coordination is still lacking. The young can crawl slightly at six and one-half days, the external auditory canal opens at 10 days, and the eyes open between 10 and 11½ days, those of the larger members of the litter opening first.

As soon as the eyes are open the young begin to leave the nest box and their attitude changes from one of apprehension to curiosity. At 11½ days the molars erupt and almost at once the young begin nibbling green vegetation. They can be successfully weaned at 13 days.

We noticed that from 14 to 28 days the young were deficient in avoidance reaction. Unlike the adults they were easy to approach and could be touched before darting away. If the same behavior is displayed by young mice in the wild it will render them more vulnerable to predation during that period.

Weight Gain

At birth the young weighed between 1.59 and 2.2 gm., with a mean of 1.7. There was no significant difference between members of large and small litters. For the first four days the weight gain averaged 0.4 gm. per day, then it increased to nearly 0.7 gm. per day until 20 days. Rate of gain declined gradually thereafter until by 50 days average nonreproductive weight was reached.

Fig. 1 illustrates the mean growth curve for the 41 young of both sexes from birth to 38 days of age. The curve can be subdivided into four different growth periods. Admittedly the shift from one rate of growth to the next is not as abrupt as is suggested by the intersection of the lines on our figure but the change is none the less real.

The coefficient of growth (K) that expresses the instantaneous growth rate based upon the formula $K=(\ln W_2-\ln W_1)/(t_2-t_1)$ has been calculated for each of the growth periods. During the first period, lasting until nine days, $K = .1617$. This is the period of nursing during which the young are blind and sedentary. By the end of nine days the milk supply is probably no longer meeting the demands placed upon it and mean instantaneous growth drops to $K = .0630$ until 20 days. The explanation for the third phase is not understood, during it $K = .0328$. Either this or the next is the pubertal break. It is pertinent to recall that at 22–26 days the external genitalia become easily differentiable. It is believed therefore that the break at 20 days is that of puberty. From 30 to 38 days instantaneous rate of gain decreases still further to .019.

It is pertinent to remark that growth data for the long inbred strain of laboratory white mice reveal almost identical instantaneous growth rates. We have calculated K for the *Mus musculus* stocks of the University of British Columbia animal unit from data kindly supplied by Dr. A. J. Wood.

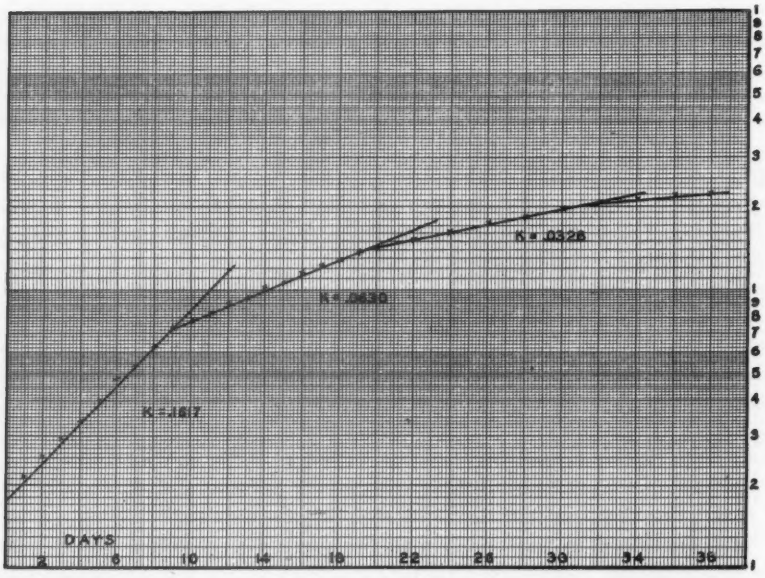

FIG. 1. Mean growth curve of 41 individuals of both sexes from birth to 38 days.

In the first phase *Mus musculus* has a K value of .1650 as against *Microtus oregoni*'s .1617. Similarly that of the second phase is .0620 in *Mus*, .0630 in *Microtus*. The *Mus* data do not include the postpubertal period.

While it is not possible, on this one comparison, to impute a basic genetically controlled growth rate for all mouse-size rodents, it is quite possible that one exists and it would be most worth while to have comparable rates calculated for additional species.

The regimen described is that operative during the most active part of the breeding season in the early summer. Litters born during the late summer and winter grew more slowly and females in such litters took as long as 60 days to reach a weight of 20-24 gm.

Age and weight were found to be closely related until 32 days after birth. Beyond that time, however, the individual variation becomes so great that inference on age from weight is invalid. Less uniform growth conditions in the wild may give more variable results.

Rate of gain was apparently similar in both sexes up to between 34 and 35 days at which time that of the females was sharply reduced. However we lack the data to elaborate on the later growth phases of either sex.

Males gained weight rapidly until active spermatogenesis commenced between the ages of 40 and 50 days; they then reached a more or less stable weight which was maintained as long as they were kept isolated from an adult female. When placed with an adult female there was a weight gain of from 5 to 10 gm. that was maintained through the breeding season. If however the male was again isolated, the weight receded to a lower level. What we regard as a typical weight history of this sort is illustrated in Fig. 2.

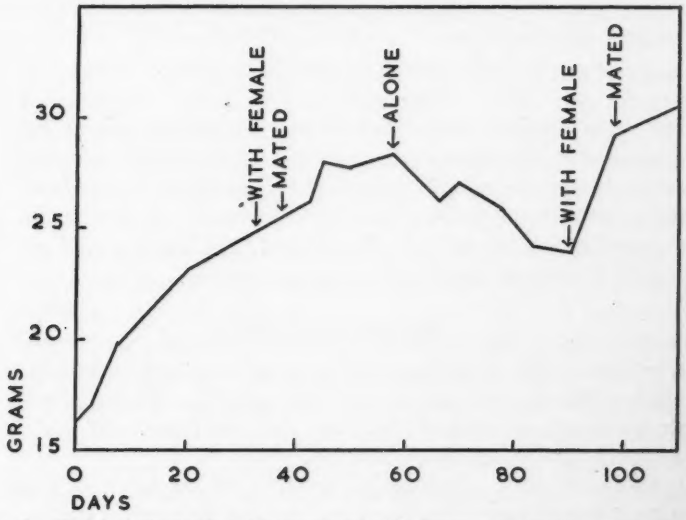

FIG. 2. Weight behavior in male associated with presence of female. Data are those of one animal chosen to represent a category.

This male was mated before it had stabilized its mature weight, and its weight increased rather rapidly about 3 gm. to 28 gm. Later, when the female was removed the male declined to a weight of 23 gm. It was then placed once again with a female and again revealed the rapid increase of weight, this time to 31 gm. This type of weight behavior was apparent in 10 males subjected to regular weighing.

Age of Sexual Maturity

It was found difficult to determine the sex of young voles with certainty until they were 22 to 26 days old. However it was thought possible that some of the females might undergo puberty prior to this age. In order to guard against the first oestrus passing unnoticed, we introduced a mature male into the cage containing the young of one or more litters. The immature males were too young to arouse fighting on the part of the adult and the animals quickly settled down. The animals were kept under the closest observation and in this way the first receptive periods of the females were determined.

As will be discussed later, the age at first oestrus was found to depend in part on the season of the year. However, for those young maturing during the active reproductive season, first oestrus occurred between 22 and 24 days following birth. Montagu (8) has called attention to the existence of a sterile period, or one of relative sterility, immediately following puberty in *Mus musculus* and in many primates. The same situation was found to exist in this *Microtus*, for, while coitus began at 22 days after birth, the earliest conception occurred at 27 days and many females did not conceive until 35–36 days old. Thus the evidence suggests a period of sterility of from 5 to 14 days following first oestrus, a period somewhat shorter than that in *Mus musculus* (30 days).

The onset of puberty in male *Microtus* was tested by placing males with adult females that had recently weaned litters and were believed to be receptive. First coitus in males took place between the ages of 34 and 38 days but none of the females served by such young animals became pregnant. Spermatozoa began to appear in the epididymis at the age of 42 days but did not become abundant until the animals were seven to eight weeks old.

Some experimental litters received restricted light budget. All females in this group were seven weeks of age before first oestrus.

Breeding Season

In early March, 1948, when the study was begun, a few females had already mated while a few had still not mated by March 15. However by April 24 all adult females captured had borne at least one litter. All males taken after March 14 were in breeding condition.

Adult females both in captivity and in the wild continued to breed until the middle of September. However later matings do occur as indicated by a young male approximately 30 days old taken on December 6. In this case

there must have been a fertile mating in mid-October. Fertile males were taken until November 23 and it seems apparent that the close of the breeding season is a function of the female.

Young females of the first litters assumed the breeding behavior of adults. The latest primiparous females were taken on July 22 and 24. Neither outdoors nor in the laboratory was there any evidence that females reaching mature weight after the period indicated by these dates took any part in the breeding activity of that year.

A similar condition applied to the males. Young males captured in the last week of July, although of the same weight as sexually active males taken two weeks previously, were not undergoing puberty. The youngest reproductive males were present during the first three weeks of June. Evidently then only the first half of the litters born in the season will breed during the same season.

In the spring of 1949 breeding apparently started in the wild earlier than in the previous year. A female that had just given birth was taken on March 10. This mating must have occurred prior to February 16. A more extreme case is that of a young animal trapped March 9. Judged by our weight curves, it was probably born in the second week of February from a mating in the third week of January.

The Influence of Light and Heat on Reproduction

In order to investigate the position of *Microtus oregoni* with respect to the influence of light on its reproductive cycle some rather limited experiments were undertaken. They were planned largely in an attempt to obtain more litters for other studies of growth and reproduction. The results cannot be thought of as conclusive but do suggest that *M. oregoni* like *M. agrestis* (3, 4) is stimulated by temperature and probably also by light.

On October 16, when time from sunrise to sunset was 10 hr. 48 min., the light budget was augmented by a progressive 3 min. nightly.

Four males and six females, all of them laboratory raised, were subjected to this additional light while two males and three females were set aside as controls, maintained at the same temperature but with only the seasonal light.

From January 20 both groups were given 14 hr. illumination daily. Both groups were maintained at a temperature of 57°–63°, thus much above the seasonal normal.

Two females had borne litters prior to the experiment. One of them was included in each group.

The six females on the increased light regimen bore 11 litters between December 5 and February 20, with a total of 31 young of which 21 lived to maturity. The control group conceived five litters while under the experimental conditions. These contained 13 young of which six lived. Animals in the wild were not breeding during this period.

TABLE I

DATES OF LITTERING OF EXPERIMENTAL FEMALES

Heat and light (6 females)	Heat only (3 females)
Dec. 5, 8, 12, 20, 21	Dec. 29
Jan. 2, 25, 26	Jan. 8, 9, 22
Feb. 2, 11, 20	Feb. 3

This inconclusive experiment reveals no significant difference between the number of litters produced by the two groups. The experimental conditions, presumably the high temperature, induced out of season breeding in both groups. The added light regimen appeared to have the effect of additional stimulation, for all but one of the illuminated animals had borne a litter before the first control animal gave birth. The very limited data suggest the need for more extensive work on microtines of different habits with respect to normal exposure to light and temperature before general conclusions can be drawn as to the nature of the stimulating factor.

In a largely subterranean species, as opposed to the more terrestrial *M. agrestis*, one might presuppose a reduced sensitivity to light response in the delicately adjusted gonadotropic pituitary mechanism and the results here suggest that this is the case.

In both groups of animals the males reacted more promptly than the females and some at least were in breeding condition one month after the light experiment was begun, and about two months after the animals were exposed to the increased temperature.

Mating

Coitus is marked by several periods of vigorous squeaking. These last for 10 to 15 min. at a time and usually recur at half-hourly intervals for as long as five hours. Copulation takes place near the beginning of the vocal periods, it is of brief duration, usually not more than two or three seconds and is repeated several times, at short intervals.

Gestation Period

Precise length of gestation period from coitus was obtained for only four of the litters. These were 23 days 8 hr.; 23 days 12 hr.; 24 days; and 24 days 12 hr. for a mean of 23 days 20 hr. This is somewhat longer than the periods given for other species of the genus. Hatfield (5) gave that of *M. californicus* as 21 days, increased to 22 by lack of exercise in caged animals; Hamilton (4) cites 21 days for *M. pennsylvanicus* and Ransom (11) the same for *M. agrestis*. However the length of the gestation period within a species and perhaps also within a genus may be related to the size of the litter (1) and this may be involved in the present instance. *M. oregoni* has smaller litters than do the other species mentioned. There was no indication of a prolonged gestation during a lactation pregnancy.

The intervals between the births of successive litters are of interest as an indication of the time of occurrence of the first oestrus after parturition and the spacing of subsequent oestrus periods. Exact intervals are available for only 11 litters. In one of these litters, the interval was 23½ days; in two it was 24, one 24½, and three 25 days; thus there were seven instances in which a postparturient oestrus resulted in pregnancy. From these intervals it might be inferred either that gestation is frequently 24½ or 25 days or that postparturient oestrus may occur during a period of 36 hr. after birth of a litter. Evidence for a shorter gestation period is fairly conclusive and favors the latter alternative.

In the remaining four cases intervals between successive litters were 37, 42, 51, and 54 days. There is thus no indication in our experience of a lactation oestrus distinct from the postparturient oestrus.

The winter anoestrus is accompanied by closure of the vulva. The vulva may or may not be closed subsequent to mating, irrespective of pregnancy.

Litter Size

Twenty-eight litters were born in captivity; they ranged in size from one to five with a mean of 2.79. The litter size as obtained from wild-caught animals, with 26 cases involved, similarly ranged from one to five but had a mean of 3.11. The mode in both groups was three and the combined mean 2.95. Litter size in the wild was based upon counts of fetuses. In captivity litters were examined, counted, and weighed within a few hours after birth. Some discrepancy in apparent litter size in the two groups may arise from the difference in basis of assessment. Resorptions may have reduced the actual number of young born to the wild stock. It is possible also that cannibalism might reduce the size of the laboratory born litters but there was no evidence of that in the group included and we had little trouble of this sort in the colony.

There is a discrepancy in the number of implantations in the two horns of the uterus. Among 78 implantations 49 were in the right horn and only 29 in the left. This is a peculiarity remarked upon in other groups of animals and is of no understood significance.

The average number of young apparently increases with successive litters through the first few born to the female. Table II gives the data available. It is unfortunately inadequate among the litters of older females.

TABLE II

NUMBER OF YOUNG IN SUCCESSIVE LITTERS

Litter number	Number of litters	Mean number in litter	Range
1	12	2.3	1–4
2	8	3.1	2–4
3	4	3.3	3–4
4	2	4.5	4–5

Only one female had more than four litters and she had two young in each of her fifth and sixth litters. Jameson (6) found that litters in *Microtus ochrogaster* averaged smaller at the beginning and at the end of the breeding season. Our data confirm his observations with respect to the latter part of the season. Thus mean litter size in the wild in June was 3.3, in July 3.1, and in September 2.5.

It is difficult to account for this decrease on a basis of change in population structure.

Sex Ratio

The sex ratio as determined from all young reaching 30 days age was 52 : 48 in favor of the males. This was a slightly lower ratio than that yielded by the 107 wild-caught animals of all ages (54 : 46). An explanation may be found in the somewhat greater activity of males rendering them more vulnerable to capture.

Reproductive Potential

It is well known that in all animals there is a notable disparity between theoretical and realized rates of reproduction. In our captive animals one female gave birth to six litters in 320 days. In no case were the young removed from the mother. It must be remembered however that breeding was artificially prolonged by the use of heat and light. Two other females bore three litters each in 131 and 132 days. In these instances the intervals between births of successive litters were 25 and 78 days for the first female and 42 and 24 for the second. In each instance it will be noted that the post-parturient oestrus was unsuccessful as often as it was successful.

Our records of wild-caught animals are inconclusive as to the number of litters normally occurring. A close study of the age classes of young in the wild population in midsummer reveals three distinct age groups, resulting from litters born about April 15, May 15, and June 15. Subsequent litters were known to occur but the irregularity of their birth times made them impossible to detect by statistical methods. It seems to us, however, that four or five litters per year constitute the usual maximum contribution by a female.

A factor of importance to reproductive potential of the species was the unexplained deaths of all but two of the primiparous litters. Subsequent litters in contrast had a high rate of survival.

Longevity

Our longevity records were spoiled by the accidental destruction of the colony. However at that time no animal of known age had died of natural causes. Two females captured as adults were still alive and in active reproduction after 320 days in the colony. A male captured as a juvenile was

active as a sire after 300 days in captivity. It would appear therefore that *M. o. serpens* in captivity has a greater life expectancy than the 260 days reported for *M. agrestis* by Leslie and Ransom (7).

The many additional hazards of life in the wild impose a reduced life expectancy upon the animals. An examination including body weight, reproductive organs, and skull, of 150 wild-caught animals taken over several years has yielded no individuals that had lived out a calendar year.

The late autumn population in each year consists of the young of the year with few exceptions.

Summary

This study of the largely subterranean vole *Microtus oregoni serpens* has revealed a number of facts about its reproductive biology.

1. The gestation period is 23½ to 25 days with a mean of 23 days, 20 hr. This is longer than that reported for any other *Microtus* and may be related to the small litter size.

2. Litters varied from one to five with a mean of 2.95 and a mode of three. This is the smallest mean litter size known in the genus and probably reflects an adaptation to the reduced vulnerability conferred by its habitat and mode of life.

3. It can be inferred from our field data that a maximum of four or five litters may be produced in a year.

4. A large proportion of first litters born in captivity died shortly after birth.

5. Weaning takes place at about 13 days.

6. A fact not previously reported for any cricetine rodent is the existence of a sterile period of 5 to 14 or more days following puberty in both sexes.

7. Females undergo first oestrus at 22–24 days if they reach that age during the first half of the breeding season. Puberty in males occurs between 34 and 38 days, but abundant sperm production does not occur before seven weeks.

8. On the basis of our data it is postulated that in both sexes only the first two or three litters produced each year reproduce that year. All animals reaching adult age and weight later than the last week of July postpone puberty until the following year.

9. A postpartum oestrus resulted in pregnancy in two-thirds of the females. It is not known whether the remainder did not experience postpartum oestrus or did not conceive in it.

10. An inconclusive experiment suggests that artificial heat alone induced breeding activity during the winter months when the wild populations were not breeding. An increased light regimen in addition to the heat may have produced a slight added stimulation detectable in more rapid response to heat plus light than to heat alone.

11. Gain in weight up to 38 days of age has been shown to fall into four rate phases. The calculated coefficient of growth (instantaneous growth rate) was .1617 for the first 9 days; .0630 between 9 and 20 days; .0328 from 20 to 30 days then falls to .019.

12. This rate of gain regimen is similar to that of the laboratory white mouse (*Mus musculus*, Linnaeus).

13. Limited data have shown an unexplained weight gain of about 20% when a male is placed with a breeding female. A similar amount of weight is lost if the pair is subsequently separated.

14. Observed sex ratio was 52 males to 48 females at 30 days.

15. Young from 14 to 28 days were deficient in fear reaction and probably much more vulnerable to predation than older young.

16. Longevity in captivity exceeds 320 days but in the wild there appears to be a complete annual population replacement. It is to be expected that occasional exceptions may occur.

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APPARATUS FOR STUDYING BEHAVIOR RESPONSES OF INSECTS TO OLFACTORY STIMULI IN STILL AIR[1]

By D. P. Pielou[2]

Abstract

Two new forms of apparatus are described for the study of olfactory responses. In the first type, insects are free to move in a diffusion gradient of vapor in still air. The gradient is stabilized by the presence of absorbent charcoal. The movements of insects are observed in relation to this gradient. In the second type, insects are exposed to an olfactory stimulus that is spatially uniform but changes in time. This is accomplished by moving a slider, containing the olfactory stimulus source, under an observation chamber with a permeable floor.

Introduction

Most laboratory work on the responses of insects to odors has been done with some form of apparatus in which moving air carries the stimulus to the insects. Olfactometers are usually designed to offer the test insects a choice to two air streams, one odorous, the other a control. The original apparatus of this type was designed by Barrows (1) in 1907, but its modern form was produced by McIndoo (7) in 1926. Important modifications making work with quantitatively defined stimuli possible were introduced by Hoskins and Craig (6) in 1934 and Weiting and Hoskins (11) in 1939. The ultimate in design of instruments of this type seemed to have been achieved by Willis (12) in 1947 and especially by Willis and Roth (13) in 1950.

Though most valuable results have been obtained with instruments of this type in the determination of the degree of chemical sensitivity of insects, it has not been easily possible to analyze the responses in terms of behavior reactions, that is, in terms of the types of sensory-muscular mechanisms responsible for orientation, 'navigation', and direction-finding in insects (Fraenkel and Gunn (3)). This is because, in the conventional moving-air olfactometer, the stimulus is twofold, consisting both of a nondirectional chemical stimulation and of a directional mechanical stimulus of air movement. The directional element of the latter makes possible an immediate directed reaction if the insect possesses sense organs of the appropriate kind.

Theoretically, a directed response of the tropotactic type (Fraenkel and Gunn (3)) is not possible in a gradient of nondirectional stimulation, such as exists around a source of diffusing chemical vapor, unless the gradient is so steep that each of the two antennae perceives a different concentration. A directed response of the klinotactic type is, however, possible in moderate gradients, as are the various types of undirected reactions (kineses).

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Analysis of the mechanisms of behavior reactions to directional stimuli such as light has been extensive (3), but there are fewer such studies concerning olfactory stimulation. For the study of this problem two basic types of apparatus have been designed.

Diffusion Gradients Stabilized by Absorbent Charcoal

The responses of insects—behavior movements such as kineses and taxes—can to some degree be investigated in the simple diffusion gradient that surrounds a source of stimulation in still air. However, as volatile material is constantly being evaporated, the air concentration is continually being changed and in a closed chamber evaporation proceeds until the equilibrium vapor pressure is reached. Gaseous concentration is then uniform and no gradient exists. In the open air, evaporation proceeds till the source is dispersed, though this may take years for some materials.

However, if a considerable surface area of absorbent charcoal is present in a closed chamber this material removes vapor locally and so tends to set up a diffusion gradient that eventually becomes stable and persists until the source is exhausted or the charcoal saturated. Experiments, of course, are usually concluded before either of these occurs.

Empirical test has shown that although various kinds of apparatus can be devised the following points must be observed. The apparatus must be shallow in proportion to its area if stable gradients are to be obtained; the height above the exposure surfaces should not be much greater than that of the insects being tested. In the apparatus constructed, for use with the parasitic wasp *Macrocentrus ancylivorus* Rohw., the height was 0.6 cm. The insects are in fact almost confined to movement in two dimensions. The area of absorbent charcoal must be large in proportion to the surface of the odor-emitting substance unless the latter is very dilute. There is a limit to the absorptive powers of charcoal, and strong odors must not be used in small chambers because the insects become so agitated that useful conclusions cannot be drawn. Extreme care must be exercised in washing and cleaning apparatus after each experiment; charcoal should be heated to redness to drive off gases or a fresh supply used in each experiment; substances such as essential oils should be confined in glass containers that can be washed satisfactorily, or, preferably, in cheap vials which can be discarded after each test.

Fig. 1 shows the construction of a typical piece of apparatus. The lid of the chamber is made of a large piece of acrylic plastic (Lucite, Plexiglass, Perspex) thick enough not to sag in the middle; the chamber walls are made of strips of the same material cemented to the edge of this. A glass lid is preferable in very critical experiments. The 'false floor' is a sheet of paper or cloth stretched taut and clamped down tightly between the chamber wall and the aluminum base-plate. It is discarded after each experiment. The source of olfactory stimulus (chemical, vegetative material, insect of sex opposite to test animal, etc.) can be removed from below without disturbing the closed chamber, as the base-plate dish is supported on legs. The base-plate itself

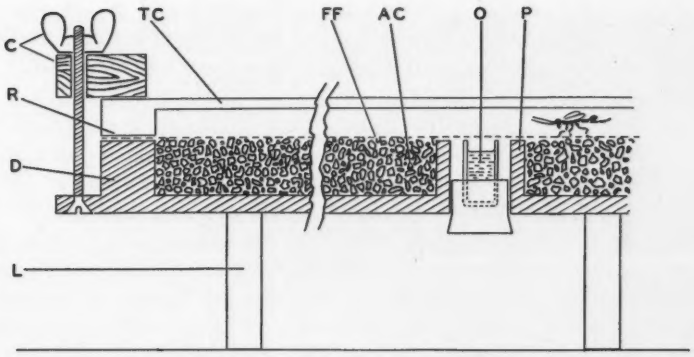

FIG. 1. Cross section of part of typical apparatus showing construction and principle. *AC*, absorbent charcoal; *D*, cast aluminum dish (base-plate); *FF*, false floor of paper or cloth; *L*, leg; *O*, odor source in vial; *P*, circular port allowing removal of bung and vial; *TC*, transparent cover and wall. The rims, *R*, of the dish and the cover, and also the extended edge of the false floor between them are clamped together tightly by the frame and screw assembly, *C*.

is made from cast aluminum; such plates can be made cheaply in a jobbing foundry accustomed to making small aluminum castings if a proper pattern in wood is provided.

Figs. 2 and 3 show the apparatus. Insects are introduced through the holes in the Lucite lids and the lids are closed with small slips of Lucite. The central port may be used alone; there will then be a circular diffusion gradient around it. This arrangement is useful for detailed observations on the movements of one insect. Another convenient arrangement is to ignore the central port and use the four outer ports—two as controls—and observe the distribution of numbers of insects with the false floor marked out into four quadrants. Fig. 4 shows the base-plate of a different form of arena, cast in the form of a number of cells. Olfactory material should not, of course, be put directly in any of the cells; otherwise cleaning will be a serious problem. Flat glass dishes should be put in the appropriate cells.

Fig. 5 shows a simple form of small choice chamber in which the distribution of insects is recorded at intervals on the right and left halves above the charcoal and stimulant respectively. In control experiments, if there is no extraneous factor such as asymmetrical lighting, distribution should be equal on the two halves. Results may be analyzed by the standard error of a proportion method. This apparatus is mainly useful in determining the limits of sensitivity in still air; it is generally too small for studies on orientation except with small, slow-moving insects. Used in this way, a number of these choice chambers can be operated at once. The apparatus has a generic relation to the humidity choice chamber of Gunn and Kennedy (4), in which sulphuric acid solutions of different concentrations set up a water vapor gradient.

A long, narrow form of gradient suitable for quantitative work is shown in Fig. 6. Its general structure is the same as that of the others. Whatever

form of apparatus is used it is important that it should be of the right dimensions, especially that it should not be too small, in relation to the size and activity of the insects. Only trial and error can show what this is.

The charcoal-stabilized diffusion gradient is particularly useful for work with ammonia, which is readily absorbed by charcoal; a great variety of insects (2) respond to very dilute concentrations of ammonia, a decomposition product of proteins. Further, it is fortunate that there are standard chemical procedures in which ammonia can be detected and measured at very great dilutions, e.g. the Nessler method. We have not adapted any method for use in estimating the actual ammonia in the air at various points in the gradient; this could certainly be done, however, if the nature of an investigation demanded an exact quantitative description of the gradient. In work on threshold values for responses this would be most desirable. At present our practice is simply to state the concentration of ammonia, etc. in solution in the vials or dishes below the false floors of the test chambers. This specifies conditions but gives no information about the concentration of vapor in the air. With the small choice chamber (Fig. 5) for instance, *Pseudosarcophaga affinis* (Fall.) responded positively, under suitable conditions, to dilutions of ammonia of one part in one million.

Apparatus for Studying Responses to Changing Intensities

In a gradient of stimulation, insects are brought into regions of different intensity by their own movements. The responses they then make cause them to aggregate at certain intensities. The analysis of these responses can often be simplified if we reverse the procedure, that is, if we keep the insects in a closed chamber with uniform stimulation throughout and then change the intensity of that stimulation quickly. This can easily be done with light, for instance, and has led (10) to a comparatively simple explanation of behavior movements that appear complex or indefinite in gradients of, or at boundaries of sharp differences in, light intensity (9, 10).

If we are to analyze behavior responses to olfactory stimulation, it is clear that the change in intensity must be brought about by diffusion alone.

Figs. 7 and 9 show apparatus in which this can be accomplished. Insects are confined on a false, permeable floor in a shallow (0.6 cm. internal height) container 35 × 35 cm. Under the false floor a long glass slider can be moved in and out to present either of two flat evaporation surfaces. These surfaces are squares of filter or blotting paper, 35 × 35 cm., attached by scotch tape to the glass, and moistened with dilute solutions of the substances being tested. The glass slider is moved very carefully so as not to disturb the insects by mechanical action. The behavior responses are noted from the time the evaporating surface is directly under the test chamber. Gross qualitative changes can be observed with a considerable number of insects present at once. For detailed analysis, however, the movements of a single insect should be watched and recorded on squared paper; marks should be made on the track

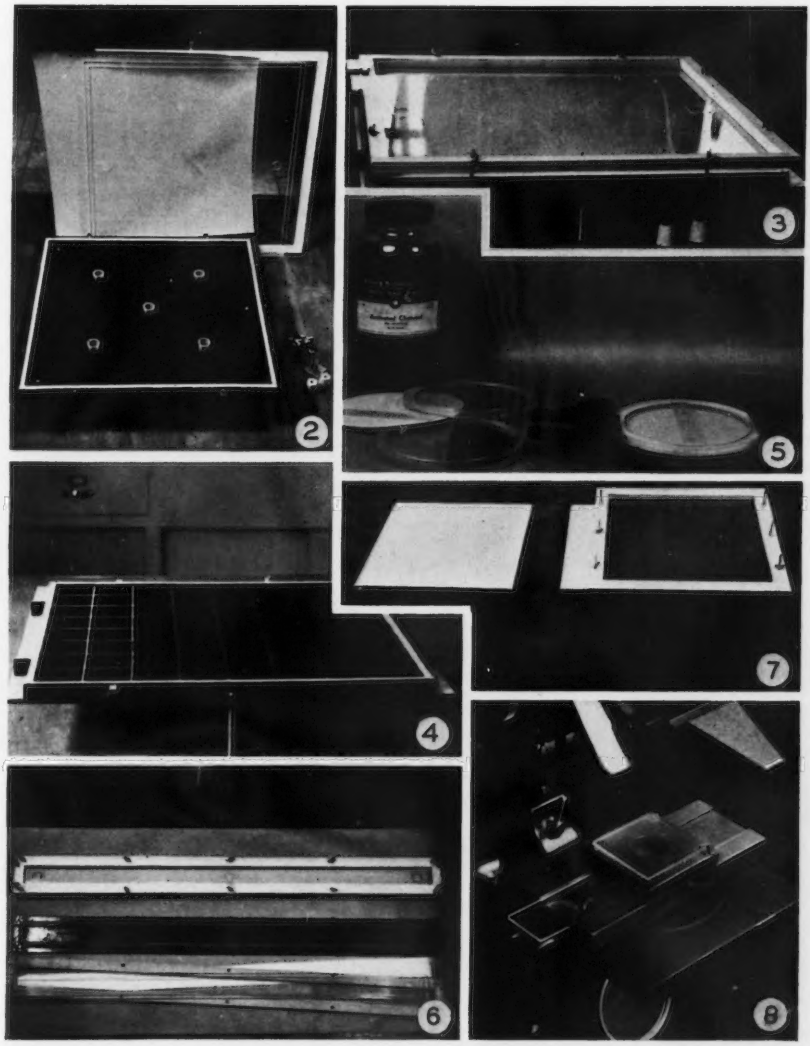

FIG. 2. Large square arena dismantled, showing circular ports on the base-plate. Odor (point source) vials, paper floor, Lucite lid, clamping frame, and tightening screws are removed. Absorbent charcoal is not shown. FIG. 3. Large square arena assembled. FIG. 4. Another form of base-plate for the square arena. The olfactory stimulus is in small flat glass dishes in one or more of the cells. The remaining cells are filled with absorbent charcoal. FIG. 5. Alternative, or choice, chamber for olfactory stimuli. Absorbent charcoal in position. Chamber on right is set up for experimentation. FIG. 6. Long form of apparatus. Nearer model is partly dismantled; one in rear set up for experimentation. FIG. 7. Apparatus for observing the effects of temporal changes in olfactory stimulus on behavior movements of a number of insects. FIG. 8. Apparatus for observing the effects of temporal changes in olfactory stimulus on single insects.

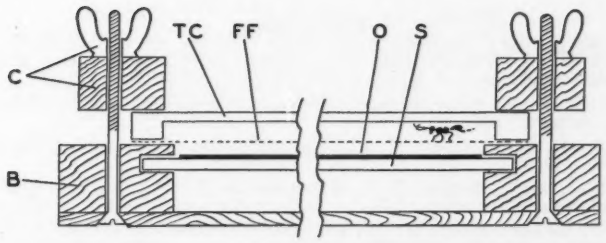

FIG. 9. Cross section of apparatus shown in Fig. 7. *B*, wooden base; *S*, sliding glass plate; *O*, odor source in filter paper on glass plate; *FF*, false floor of permeable paper or cloth; *TC*, transparent cover and lid; *C*, clamp and screw assembly.

to indicate time at half-minute intervals from the commencement of observation. Tracks of this sort are invaluable in analyzing the mechanism of insect behavior responses (10).

This apparatus has also given good results in tests of behavior responses to humidity. For such tests one of the filter squares is soaked in distilled water and the other is dry, or is soaked in a salt solution giving a desired, lower humidity. Undesiccated adults of *Tenebrio molitor* L., a species very sensitive to differences in humidity (8), responded well and it may be possible to analyze the response more fully than has been done previously (5).

A smaller type of apparatus based on the same principle is shown in Fig. 8. This apparatus is mainly useful in establishing the lower threshold of sensitivity in insects to dilute odors—at least as far as is apparent from any visible reaction such as movement of the antennae. A single insect is confined in a metal cell 2 cm. in diameter and 1 cm. deep. The cell is closed above with a glass slip; the bottom is a false floor of wire screen or gauze. Cavity slides, containing small quantities of odorous material in the cavities, can be slipped under the latter. A double-cavity slide is shown in Fig. 8 but it is generally better to use each test material, including the control, on a separate single-cavity slide so as to prevent odor sources being in close approximation. With this apparatus tests have shown that *Macrocentrus ancylivorus* Rohw. is sensitive to extremely dilute solutions of ammonia.

Acknowledgments

The basic design of apparatus in which absorbent charcoal is used was worked out by the writer in 1939 at the University of Birmingham, England; thanks are due to Prof. H. Munro Fox, F.R.S., and Dr. D. L. Gunn for encouragement. Acknowledgment is made to the Beit Trustees for the award of a postdoctorate research fellowship to continue the work at the Imperial College of Science, London; this project was, however, soon abandoned on the outbreak of war. The equipment described was not made till the facilities of the Belleville laboratory became available to the writer; thanks are due to technicians R. C. Hewson, T. H. Stovell, C. F. Nicholls, and C. E. Rouse for assistance.

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HELMINTH PARASITES OF MICE IN NORTHEASTERN QUEBEC AND THE COAST OF LABRADOR[1]

By G. A. Schad[2]

Abstract

Nineteen helminths are recorded from mice of the families Cricetidae and Zapodidae in Northeastern Quebec and the coast of Labrador. The possible synonymy of *Quinqueserialis hassalli* with *Q. quinqueserialis* is discussed, *Andrya bairdi* n. sp. is described, and *Catenotaenia linsdalei* is made a synonym of *C. dendritica*.

Introduction

During the summer of 1951, Mr. D. J. Osborn of the Department of Zoology, McGill University, collected small mammals at Menihek and Knob Lake, Labrador. The following summer (1952), Messrs. Osborn, Bleakney, and Schad, of McGill University, undertook an expedition to northeastern Quebec and the coast of Labrador. Collection sites were chosen so as to extend from the north shore of the gulf of the St. Lawrence River to Ungava Bay in order that the expedition could survey many of the natural habitats found in this region and obtain information on northward distribution. The localities surveyed were Seven Islands, Mile 134 (on the Seven Islands – Knob Lake railway), Knob Lake, Fort McKenzie, Fort Chimo, and Schnack Cove (see Fig. 1).

This paper reports the helminths recovered from mice collected by both the 1951 and the 1952 expeditions. Those from the first expedition were recovered from formalized viscera and some of these were poorly fixed, contracted, and unsatisfactory for study. During the summer of 1952 helminths were recovered in the field: trematodes and cestodes were fixed in Bouin's fixative and nematodes in hot 70% alcohol. The latter collection provided more adequate material for study.

Although 238 mice of the families Cricetidae and Zapodidae were surveyed, it will be necessary to examine more material before a thorough understanding of the helminth fauna of these animals in northeastern Canada is possible.

All species collected are recorded in the summary given at the end of this paper, but only those species of parasite requiring discussion are included in the test.

Trematoda

The trematodes found in this survey are represented by two genera found in Eurasian and North American rodents, namely *Quinqueserialis* (Notocotylidae) and *Plagiorchis* (Plagiorchidae).

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[2] *Carnegie Arctic Scholar.*

THE GENUS *QUINQUESERIALIS* SKWORZOW, 1935

Quinqueserialis quinqueserialis Barker and Laughlin, 1911

Three trematodes, one from the caecum of the meadow vole, *Microtus pennsylvanicus*, and two from the small intestine of the jumping mouse, *Zapus hudsonius*, were collected. In the author's opinion, mice constitute abnormal hosts for these trematodes and thus the recovery of only three trematodes is significant.

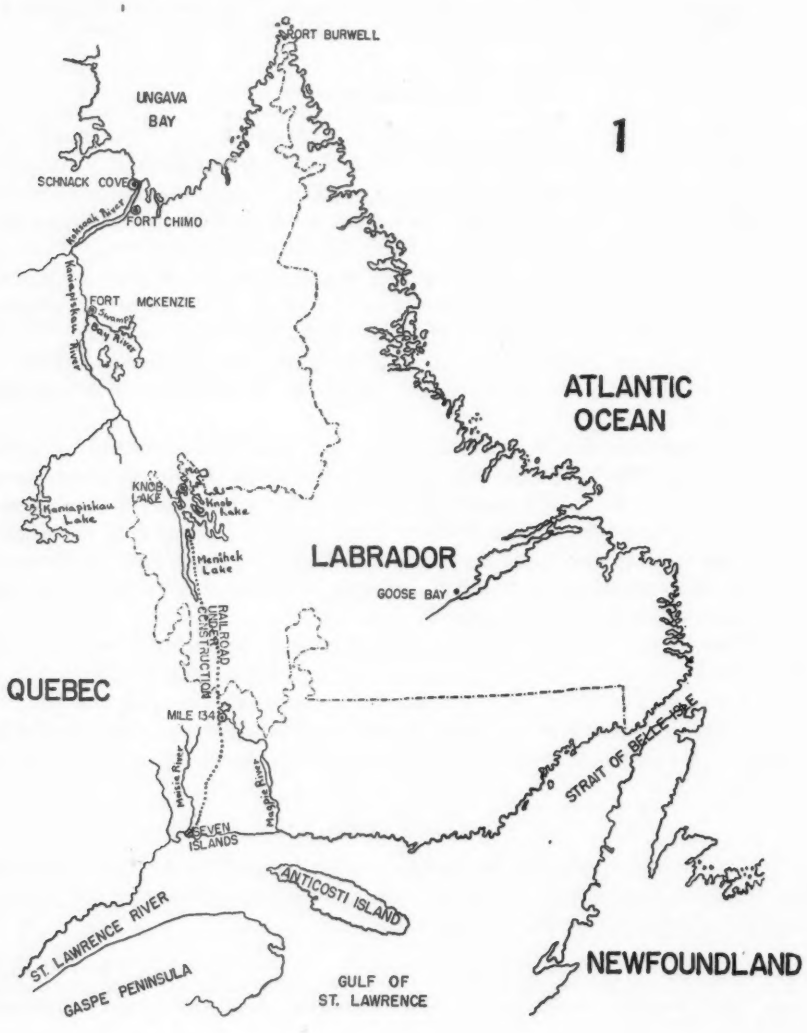


FIG. 1.

Discussion

Until recently, *Quinqueserialis hassalli* (McIntosh and McIntosh, 1934) had been considered the member of this genus parasitic in North American mice, but Rausch (8) reported a morphological intergradation between this species and *Q. quinqueserialis* and regarded his specimens from Alaskan voles as the latter.

There are only a few reports of *Q. hassalli* in the literature. These originate from widely separated areas and all record this parasite as being restricted to mice and as occurring in small numbers (3, 4, 6). Apparently the host–parasite relationship is not a normal one. *Q. quinqueserialis*, on the other hand, recently reported from mice and morphologically intergrading with *Q. hassalli*, has long been recognized as a common parasite of muskrats; it is numerous in these animals throughout their range and this host–parasite relationship can be regarded as a normal one.

It appears, therefore, that *Q. hassalli* and *Q. quinqueserialis* belong to the same species, namely *Q. quinqueserialis*, a parasite of muskrats, which is incidental in mice. It seems unlikely that two distinct species, separable only on the basis of egg size and shape, would parasitize the same host animal. Even if these criteria were valid, the incidence in mice is so low that the species could not attain the critical minimum population necessary for survival, if mice were the normal hosts.

The author is not willing to suppress the species *Q. hassalli*, but on the basis of Rausch's findings and the opinions expressed in this paper, he desires to endorse Rausch's suggestion that life history studies be carried out to determine the validity of this species.

Cestoda

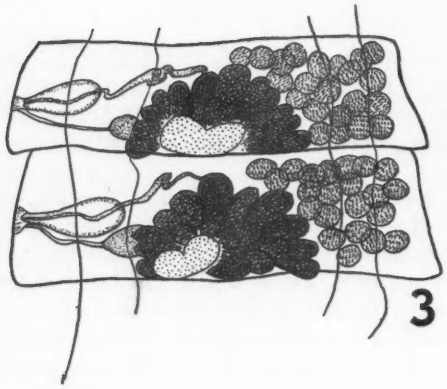
THE GENUS *ANDRYA* RAILLIET, 1915

Andrya bairdi sp. nov. (Figs. 2-5)

A series of rock voles, *Microtus crotorrhinus*, collected at Seven Islands and Fort McKenzie, Quebec, were parasitized by a cestode described here as *Andrya bairdi* sp. nov.

Diagnosis

With characters of the genus. The strobila is about 200 mm. long; maximum width is attained in gravid segments not yet containing shelled eggs. These segments may be 2 mm. in width. Mature segments are broader than long. The scolex measures about 300μ in diameter and bears suckers measuring 21μ. The genital pores are unilateral, dextral, and are located near the mid-point of the segmental margin. The cirrus pouch varies from 150 to 250μ in length and from 60 to 100μ in width. An internal seminal vesicle is present and the cirrus is armed. There is no prostate gland. A loop of the vas deferens functions as an external seminal vesicle; it may be seen dilated and sperm-filled, persisting in posterior segments. The testes, 31 to 35 in number, measure from 40 to 80μ and occupy the antiporal region of the segment from the ovary to beyond the ventral longitudinal



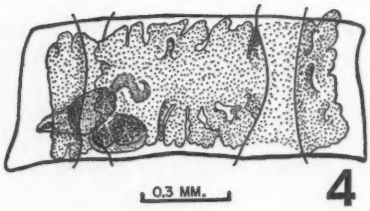


FIG. 2. Scolex of *Andrya bairdi*.
FIG. 3. Mature segments of *A. bairdi*.
FIG. 4. Early gravid segment of *A. bairdi*.
FIG. 5. Ova of *A. bairdi*.

excretory canals. The vagina, lying posterior to the cirrus pouch, passes dorsal to the ventral longitudinal excretory canal and opens into the genital atrium just posterior to the cirrus pouch. There is a prominent, oval, seminal receptacle. The ovary, medially situated, measures 250 to 400μ in width, while the prominent vitelline gland, measuring 200 to 300μ, is porally situated. The development of the uterus is reticulate and contains spherical ova 40μ in diameter. The gravid segments, with persistent excretory ducts, present a serrate appearance.

Type host:	Rock vole, *Microtus crotorrhinus*.
Type locality:	Seven Islands, Que., Canada.
Habitat:	Small intestine.
Type specimen:	U.S. National Museum, slide No. 48758.
Paratypes:	U.S. National Museum, slide No. 48759, and additional material at the Institute of Parasitology, Macdonald College, Que., Canada.

Discussion

Andrya bairdi differs from all described North American species other than *A. primordialis* Douthitt, 1915, in having unilaterally arranged genital pores. It differs from *A. primordialis*, as described by Douthitt (2), in the absence of a prostate gland. However, Rausch (8) considers the status of *A. primordialis* indefinite and points out that he has never observed a prostate gland in any of the hundreds of *Andrya* spp. that he has examined. He has deposited a specimen (slide 47801) in the U.S. National Museum which, in his opinion, may represent *A. primordialis* but which does not have a prostate gland. Furthermore, the author has in his collection some specimens from red-backed mice which have unilateral genital pores but which lack a prostate gland.

At present the author's material is insufficient to clarify the position of the North American *Andrya* having unilateral genital pores.* However, specimen 47801 of the U.S. National Museum collection resembles the author's material from red-backed mice more closely than it does *A. bairdi*. The latter differs mainly from *Andrya* sp. collected from red-backed mice in that the testes in mature segments are not confined to the area between the longitudinal excretory ducts.

It is probable that the North American *Andrya* possessing unilateral genital pores may be shown to be conspecific when an adequate series of specimens is examined, as Douthitt (2) could have been in error in his observation of prostate gland. Under present conditions, however,—that is until the description of *A. primordialis* is modified—it is necessary to describe *A. bairdi* as a new species.

THE GENUS *CATENOTAENIA* JANICKI, 1904

Several species of *Catenotaenia* have been reported from African, Eurasian, and North American rodents. Three species, *C. reggiae* Rausch, 1951, *C. dendritica* Goeze, 1782, and *C. linsdalei* McIntosh, 1941, have been recorded in North America; of these *C. dendritica* is widely distributed in the northern hemisphere (5, 8).

Catenotaenia dendritica Goeze, 1782

This cestode, the commoner tapeworm of red-backed mice in the areas surveyed, has recently been reported from red-backed mice in Alaska and the state of Washington (8). A similar cestode, *C. linsdalei* McIntosh, 1941, was described from California pocket mice and kangaroo rats. Voge (11) has given further morphological information on this species. In the author's opinion, *C. linsdalei* is a synonym of *C. dendritica*. Table I compares the measurements of these species as given by Joyeux and Baer (5), Yamaguti (12), McIntosh (7), and Voge (11).

** See Rausch (8) for a complete review of the taxonomic status of A. primordialis and a proposed method of solving the systematic problem of the Andrya with unilateral genital pores.*

TABLE I

MEASUREMENTS OF *Catenotaenia dendritica* AND *Catenotaenia linsdalei* BY VARIOUS AUTHORS

(All measurements in mm.)

	Catenotaenia dendritica by Joyeux & Baer (5)	*Catenotaenia dendritica* (= *Catenotaenia ris*) by Yamaguti (12)	*Catenotaenia linsdalei* by McIntosh (7)	*Catenotaenia linsdalei* by Voge (11)
Length	100–150	Over 120	135	95–164
Maximum width	1.5	Up to 2.5	1.0	—
Scolex diameter	0.29	0.27–0.33	—	0.11–0.22
Sucker diameter	0.15	0.11–0.16	—	0.06–0.11
Testes				
Number	140–190	140–190	130	90–150
Size	0.05	Up to 0.1	0.05–0.07	0.03–0.08
Cirrus pouch				
Length	0.14–0.18	0.12–0.18	0.14	0.12–0.17
Width	0.05–0.07	0.07–0.09	0.07	—
Uterine branches	35–40	30–49	40–50	—

Discussion

From a comparison of the figures given by Joyeux and Baer and by McIntosh there appear to be differences in the structure and position of the reproductive organs. These differences can be resolved by the fact that the mature segment studied by Joyeux and Baer was in a contracted state whereas that studied by McIntosh was not. The material collected by the author shows a variation from the crowded field of laterally ellipsoid testes as figured for *C. linsdalei* to an extended field of separate, longitudinally ellipsoid testes resembling the figure of *C. dendritica* given by Joyeux and Baer. These characters are dependent on the relative contraction of the specimens, and as stated by Rausch (8):

"In various anoplocephaline genera, including *Catenotaenia*, the lack of constant characters (for example, rostellar hooks) makes it necessary to pay particular attention to variation when specific differentiation is attempted. In *Catenotaenia* it would seem particularly advisable to take into consideration the state of strobilar contraction, since the relative positions of organs are often greatly influenced by this. Any lot of material consisting solely of strobilae either uniformly contracted or relaxed could convey an impression which might lead to an altogether erroneus conclusion in regard to specific characterization."

In the author's opinion, there is insufficient difference between *C. dendritica* and *C. linsdalei* to warrant separation and thus *C. linsdalei* is considered a synonym of *C. dendritica*, the latter name, having priority, being retained.

LARVAL CESTODES

A number of larvae of *Taenia tenuicollis* were found in the liver of red-backed mice. In addition, however, another larval form, which differed in the morphology of the hooks but not in their number and size, was recovered free among the coils of the small intestine.

Superficially, the larval bladder appears as an elongate double structure, the two parts of which are expanded distally and then tapered to form a narrow isthmus at their common middle (Fig. 6). The bladder was flattened when recovered but this may not be the natural condition. It measures 8.1 mm. in length and its greatest width is 1.4 mm. It contains two invaginated scolices at the distal expanded portions. These scolices possess a double rostellum of 42 hooks and four suckers; the hooks measure 0.16 to 0.18 mm. in length (Fig. 7). Recently, Shiller (10) reported interconnected cysticerci of *T. polycantha* from the abdominal cavity of a tundra vole. In gross appearance these are very similar to the larval forms reported here but they differ greatly in the size and shape of the hooks.

Nematoda

THE GENUS *NEMATOSPIROIDES* BAYLIS, 1926

Two species of this genus are represented in this collection, namely, *Nematospiroides carolinensis* Dikmans, 1940 and *N. dubius* Baylis, 1926. *N. carolinensis* was found in red-backed mice and specimens identified as *N. dubius* were collected from rock voles. *Nematospiroides* spp. are widespread in their occurrence in North American mice.

Nematospiroides carolinensis Dikmans, 1940 (Figs. 8, 9)

The most common parasite of the red-backed mice surveyed, *N. carolinensis*, was described by Dikmans (1) from limited material. For this reason, morphological data will be presented here.

Diagnosis

Long, filiform worms, spirally wound anteriorly. The females are almost twice as long as the males and usually more coiled. The females measure from 10.49 to 11.79 mm. in length, and the males from 6.42 to 6.50 mm. In maximum width the measurements are 0.12 to 0.19 and 0.12 to 0.15 mm., respectively. The inflated cuticle surrounding the head is marked by fine striations. Beginning immediately behind the cephalic cuticular swelling, the cuticle shows coarser striations extending longitudinally to the posterior. The esophagus leads posteriorly from a simple mouth and measures 0.47 to 0.54 mm. in males and 0.52 to 0.56 mm. in females.

Female

The reproductive system is as described for the type. The single ovejector measures 0.14 to 0.20 mm. in length and is situated 0.49 to 0.61 mm. from the posterior end, and leads to a long vagina. The tail may be

shorter than the type, measuring 0.04 to 0.06 mm.; the tail spikes measures 0.01 mm. in length and the eggs, sometimes smaller than the type, vary from 0.05 to 0.06 by 0.03 to 0.04 mm.

Male

The male is in agreement with the original description except in total length (as given below) and in the following characters: the spicules are of normal sclerotization and are not weakly chitinized as was described by Dikmans (1). The dorsal ray of the bursa differs from that described in having longer internal branches (Figs. 7, 8).

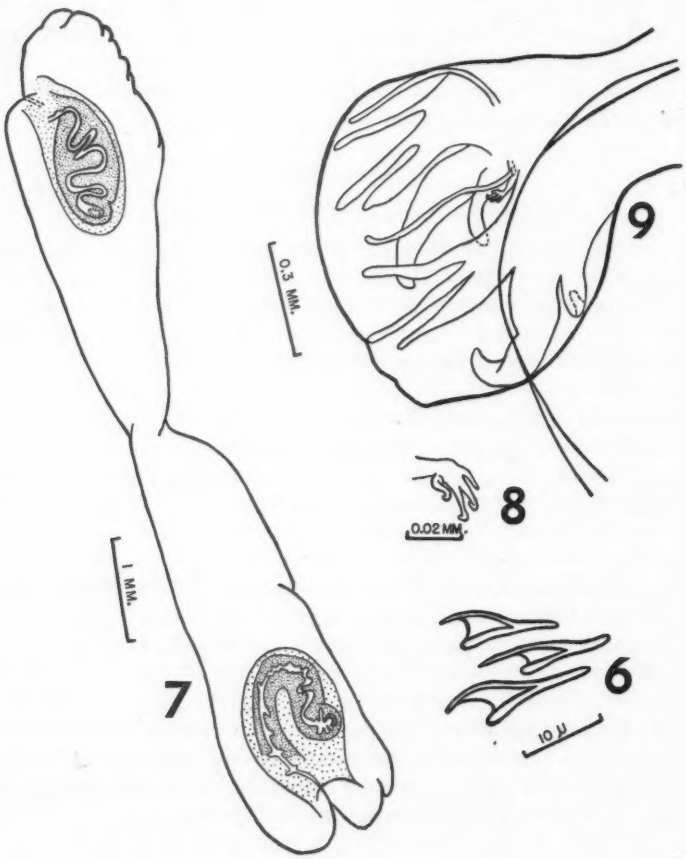


FIG. 6. Hooks of larval cestode.
FIG. 7. Larval cestode.
FIG. 8. Detail of dorsal ray shown in Fig. 9.
FIG. 9. Bursa of *Nematospiroides carolinensis*.

THE GENUS *SYPHACIA* SEURAT, 1916

This species was collected in small numbers from two voles, *Microtus pennsylvanicus;* one vole was parasitized by a single specimen only. The scarcity of members of the genus *Syphacia* in this survey is unexpected. None of the 18 deer mice, *Peromyscus*, harbored the usually ubiquitous *S. peromysci* Harkema, 1936; in an earlier survey of *Peromyscus maniculatus gracilus* at Ste. Anne de Bellevue, Que., 84% of the hosts were found to be parasitized by *S. peromysci.* Rausch (8) found *S. obvelata* abundant in Alaskan voles. Therefore, the northern location of the areas surveyed, in itself, does not seem to be responsible for the scarcity of these nematodes. The author can offer no explanation for their almost complete absence.

SUMMARY

Host	No. examined	No. parasitized	Parasite
Peromyscus maniculatus	18	1	*Taenia tenuicollis* (larvae)
Synatomys borealis	16	1	*Taenia tenuicollis* (larvae)
Synatomys borealis		9	*Paranoplocephala infrequens*[1]
Dicrostonyx hudsonius	17	2	*P. variabilis*
Dicrostonyx hudsonius		11	*Heligmosomum hudsonius*
Clethrionomyx gapperi	76	2	*Plagiorchis* sp.
Clethrionomyx gapperi		2	*Taenia tenuicollis* (larvae)
Clethrionomyx gapperi		1	Larval cestode
Clethrionomyx gapperi		7	*Andrya* sp.
Clethrionomyx gapperi		2	*Andrya macrocephala*
Clethrionomyx gapperi		18	*Catenotaenia dendritica*
Clethrionomyx gapperi		12	*Hymenolepis horrida*[2]
Clethrionomyx gapperi		7	*Nematospiroides carolinensis*
Clethrionomyx gapperi		2	*Mastophorus muris*[3]
Microtus pennsylvanicus	73	2	*Plagiorchis muris*
Microtus pennsylvanicus		1	*Quinqueserialis quinqueserialis*
Microtus pennsylvanicus		1	*Taenia tenuicollis* (larvae)
Microtus pennsylvanicus		10	*Andrya macrocephala*
Microtus pennsylvanicus		15	*Paranoplocephala infrequens*[1]
Microtus pennsylvanicus		2	*P. variabilis*
Microtus pennsylvanicus		2	*Syphacia obvelata*
Microtus crotorrhinus	14	3	*Andrya bairdi*
Microtus crotorrhinus		1	*A. macrocephala*
Microtus crotorrhinus		5	*Nematospiroides dubius*
Zapus hudsonius	20	8	*Plagiorchis proximus*
Zapus hudsonius		1	*Quinqueserialis quinqueserialis*
Zapus hudsonius		1	*Mesocestoides* sp.
Zapus hudsonius		1	*Mastophorus muris*[3]
Napaeozapus insignis	4	0	No parasites

[1] *P. infrequens, found only in light infestations, usually one or two worms per host, was restricted to the caecum in Synatomys, but occurred in both the caecum and the small intestine in Microtus.*

[2] *Although H. horrida has been recovered from many different hosts, this survey found the tapeworm restricted to Clethrionomyx. In northeastern Canada, H. horrida has now been recorded from Seven Islands and Fort McKenzie, Que. (this survey), and Godbout, Que. (Schiller (9)).*

[3] *Rausch (8) noted that, in the western Arctic, M. muris was confined to hosts in spruce forest areas. The author recovered this parasite from but three mice, all three of which were trapped in one valley heavily forested with spruce.*

Acknowledgments

The author wishes to thank Colonel P. D. Baird, Director of the Arctic Institute of North America, Montreal, Que., for his assistance and advice concerning the expedition, and Dr. T. W. M. Cameron for his guidance and helpful criticism in the preparation of this paper.

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THE EFFECTS OF THE CARCINOGEN, 2-ACETYLAMINOFLUORENE, ON LARVAL AND ADULT FROGS[1]

By David J. McCallion

Abstract

2-Acetylaminofluorene is a powerful carcinogen capable of inducing a variety of benign and malignant neoplasms in several species of animals and is required only in very small amounts for relatively short periods of time. It is somewhat soluble (1.3 mgm. per 100 cc.) in water. Frog tadpoles were raised in solutions of various strengths. Even dilute solutions proved to be highly toxic to these animals. In those animals that survived it was found that 2-acetylaminofluorene inhibits both total body growth and rate of tail regeneration; that the tadpoles remained dark (melanophores dilated) even on a light background; that lesions of the liver developed resembling those described in mammals resulting from treatment with the same and other carcinogens. It is not certain that these lesions are neoplastic, but they may be of the type that precede or accompany liver cell carcinoma. This carcinogen was also fed to or injected into adult frogs. Even small amounts were toxic to them and only a small number survived for more than a few days. In the survivors, after six to eight weeks, lesions of the liver, including necrosis and cirrhosis, appeared. No distinct neoplasms were produced.

Introduction

The occurrence of tumors, both spontaneous and induced, in Amphibia has not been widely investigated, and very little experimentation (except for the extensive study of the frog kidney carcinoma (39) by Lucké and Schlumberger) has been carried out in this field. Many of the reports in the literature concern the discovery of single individuals bearing tumors. Many of these growths have been shown not to be neoplastic at all (40). Relatively few attempts have been made to induce tumor formation in this class of animals, and only one attempt in the frog tadpole has been reported (17). No tumors have been produced in the Anura, and the lesions that have been produced in the Urodela have not been proved to be neoplastic, either benign or malignant. In a recent review of the literature, Lucké and Schlumberger (40) have considered all cases of neoplasia in the Amphibia. They have come to the conclusion that "no convincing evidence has been produced that true neoplasia has been induced by any of the chemicals used in any cold-blooded vertebrate".

For these reasons many problems associated with amphibian cancer remain unsolved. These problems include the relationship between capacity for regeneration and susceptibility to tumor formation; the relationship between regenerative processes and neoplastic processes; whether tumor tissue can be dedifferentiated and redifferentiated in a regeneration field; and whether a truly neoplastic change can be induced in amphibian tissue by chemical carcinogens.

[1] *Manuscript received in original form June 17, 1953, and, as revised, March 8, 1954.*

Contribution from the Perry Biological Laboratories, Acadia University, Wolfville, N.S. This paper includes the major content of a Ph.D. thesis submitted to Brown University, Providence, R.I. A large part of this work was supported by a grant from theNational Cancer Institute of Canada.

The present investigation represents a study of the effects of a known carcinogenic substance upon both larval and adult frogs in an attempt to produce neoplasia in these animals.

Materials and Methods

The carcinogen used in this investigation was 2-acetylaminofluorene (Eastman Kodak Co.). It is capable of inducing a variety of benign and malignant neoplasms in several species of animals. It is required in very small amounts for relatively short periods of time. It is somewhat soluble in water, 1.3 mgm. dissolving in 100 cc. (12, 56). In some instances 2-aminofluorene was used. It is similar in its action but is more soluble in water (3.0 mgm. per 100 cc.).

Large mature *Rana pipiens* obtained from the Carolina Biological Supply Co. were used in the production of eggs. Large numbers of eggs may be obtained and successfully fertilized by the method of Rugh (49) from September to April. The eggs thus obtained were raised in finger bowls, about 20 eggs in each, in tap water at room temperature. When the tadpoles were well established (three to four weeks of age) treatment with the carcinogen was begun.

Even under uniform environmental conditions the tadpoles arising from a given egg mass show considerable variation in their degree of development at any given time (46, 53). For this reason only tadpoles of approximately the same size and at the same stage of development were selected for treatment with the carcinogens and as controls.

Tadpoles were maintained in glass finger bowls such that there was about 100 cc. of solution for each animal. The solutions were changed every other day, and, at the same time, the finger bowls were washed in warm soapy water. Each tadpole, therefore, had the opportunity of absorbing 3-4 mgm. of the carcinogen per week. The tadpoles were fed fresh canned spinach in small quantities each day. This diet was supplemented with canned liver and pablum. In the same manner control tadpoles were raised in tap water and fed the same diet.

Mortality was very high among the experimental animals and although several hundred tadpoles (594) were used in these experiments only 56 survived for two weeks or longer. Since the principal objective of this investigation was to maintain a sufficiently large number of animals for a long enough time to allow tumors to develop, animals were killed sparingly and only a small number of animals were sacrificed at representative times. All animals that died were autopsied under the dissecting microscope, and in most cases the liver was studied microscopically.

Whenever tadpoles were sacrificed the body cavity was opened and the viscera examined under a dissecting microscope, then the liver was dissected out whole and fixed. In some cases the head was sectioned serially for examination of the thyroid gland. Tissues were fixed in Bouin's fluid, dehydrated through dioxane, and embedded in paraffin. The sections were stained with hematoxylin and eosin or with Mallory's connective tissue stain.

For purposes of attempting to prolong the life of liver tissue from animals treated with the carcinogens, and to study the behavior of liver tissue small pieces of liver were transferred to the body cavity of normal young larvae. The livers were dissected out of treated larvae and cut in half. One half was fixed in Bouin's fluid for microscopic examination and the other half cut into small fragments (1-2 mm. in diameter) which were inserted into the body cavity of host larvae. From time to time these grafts were recovered and prepared for microscopic examination.

During the course of this investigation a total of 150 adult frogs were treated in the following ways: 2-acetylaminofluorene or 2-aminofluorene were fed as crystals in gelatin capsules; fed mixed with ground beef; fed in gelatin capsules as various concentrations in olive oil; or injected into the dorsal lymph sac as various concentrations in olive oil or in propylene glycol. These substances were highly toxic to frogs, even in small amounts, and only three frogs (in which 2-acetylaminofluorene had been administered orally) survived longer than one week. Pieces of liver tissue from these animals were fixed in Bouin's fluid and stained with hematoxylin and eosin or Mallory's connective tissue stain.

Experiments and Results

Effects of 2-aaf on Eggs and Young Embryos

Solutions of 2-acetylaminofluorene were prepared (by diluting a saturated solution) such that 100 cc. of solution contained 1.3, 0.65, and 0.32 mgm. of the carcinogen. Similar solutions of 2-aminofluorene containing 3.0, 1.5, and 0.75 mgm. of the carcinogen per 100 cc. were prepared. Frog eggs were continuously exposed to these solutions from one hour after fertilization, from early yolk plug stage, and from late yolk plug stage. Within a few hours from the time the eggs were placed in the solutions, the layers of gelatin surrounding the eggs became light tan colored indicating that the gelatin was concentrating the dissolved substances. It is probable, therefore, that the developing eggs were exposed to higher concentrations of these substances than the original solutions indicate.

Frog eggs subjected continuously to solutions of fluorene compounds from one hour after fertilization usually survive for a very short time and not over four or five days. The effects of the fluorene compounds on the development of the eggs were not constant or related to the concentration. In general the rate of cleavage was retarded and the cleavage pattern was irregular. For example: one side of the egg divided nearly normally while cleavage on the other side was almost completely retarded; or, the animal hemisphere divided rapidly while the vegetal hemisphere was almost completely retarded. Some eggs developed as far as the yolk plug stages and a few as far as the neurula stage. Their rate of development was retarded and extensive abnormalities developed. These were: exogastrulae, hemiembryos of varying degrees of abnormality, hemigastrulae, and neurulae with extruding yolk plug. Degeneration of these embryos followed, with cells becoming rounded and

swollen and breaking away from the embryo. Degeneration began in the regions of greatest embryonic activity, i.e., at the dorsal lip of the blastopore and along the neural folds.

Young embryos subjected to these solutions in the early yolk plug stages developed exogastrulae and failed to survive. Embryos that had reached late yolk plug stage before being subjected to the solutions survived for a longer time. Many of them developed nearly normally but with their rate of growth appreciably retarded. At later stages, however, the survivors developed abnormalities of the digestive tract, probably owing to faulty absorption of the yolk mass into the floor of the gut.

Toxic Effects of 2-aaf on Frog Tadpoles

Frog tadpoles immersed in solutions of 2-aminofluorene died within 12 hr., therefore, only solutions of 2-acetylaminofluorene were used in these experiments. Although this substance has been shown to be nontoxic to mammals under various conditions (57) the mortality of frog larvae exposed to solutions of the chemical was very high. Of almost 600 tadpoles fewer than 50 survived beyond 60 days of treatment, and only 8 survived beyond 100 days of treatment. One tadpole survived for 133 days in a solution of 0.65 mgm. 2-acetylaminofluorene per 100 cc. Control animals raised in the laboratory thrived and many were raised through metamorphosis.

Effects of 2-aaf on the Growth of Frog Tadpoles

Forty tadpoles obtained from the same egg mass and of the same total length were divided into 4 groups of 10 animals each. One group was raised in a solution of 2-acetylaminofluorene of 1.3 mgm. per 100 cc., a second group in a solution of 0.65 mgm. per 100 cc., and a third group in a solution of 0.32 mgm. per 100 cc. The fourth group was raised in tap water. The total length of the tadpoles in each group was measured at several times, and at the same time their stage of development was noted (according to Taylor and Kollros (53)). Although all the animals were the same length at the beginning of exposure to the carcinogen a considerable discrepancy in length soon appeared (Fig. 1). After 50 days' exposure to the carcinogen the average lengths of these tadpoles (with standard deviations) were 28.67 $\pm$ 2.02 mm., 30.0 $\pm$ 2.94 mm., and 27.4 $\pm$ 1.14 mm. respectively. At the same time the average length of the animals raised in tap water was 36.0 $\pm$ 2.89 mm. After 67 days of treatment the average lengths of the experimental tadpoles were 38.0 $\pm$ 2.83 mm., 38.2 $\pm$ 3.37 mm., and 38.6 $\pm$ 3.21 mm.

FIG. 1. Photograph of control and treated tadpoles to show difference in size. The animals on the right were exposed to a 1.3 mgm. per 100 cc. solution of 2-acetylaminofluorene for 40 days. Those on the left were the same age and raised in tap water. $\times$1.75.

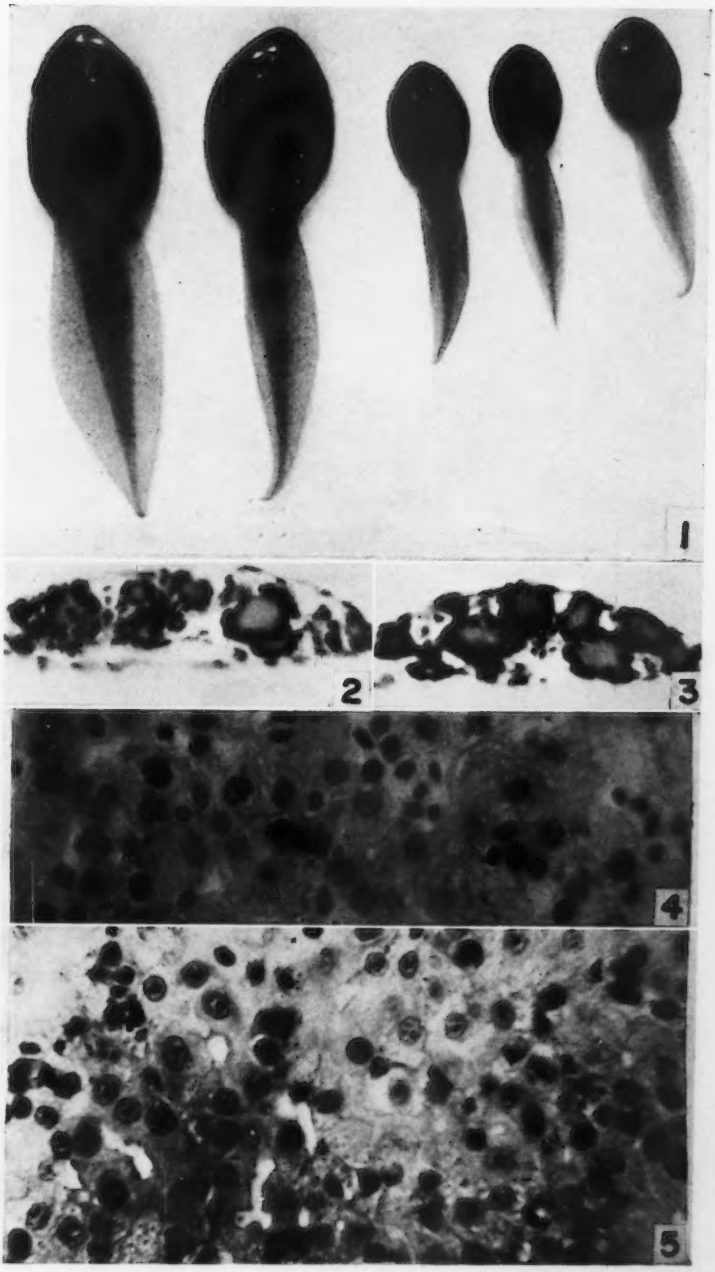
FIG. 2. Section of the thyroid gland of a control tadpole. $\times$230.

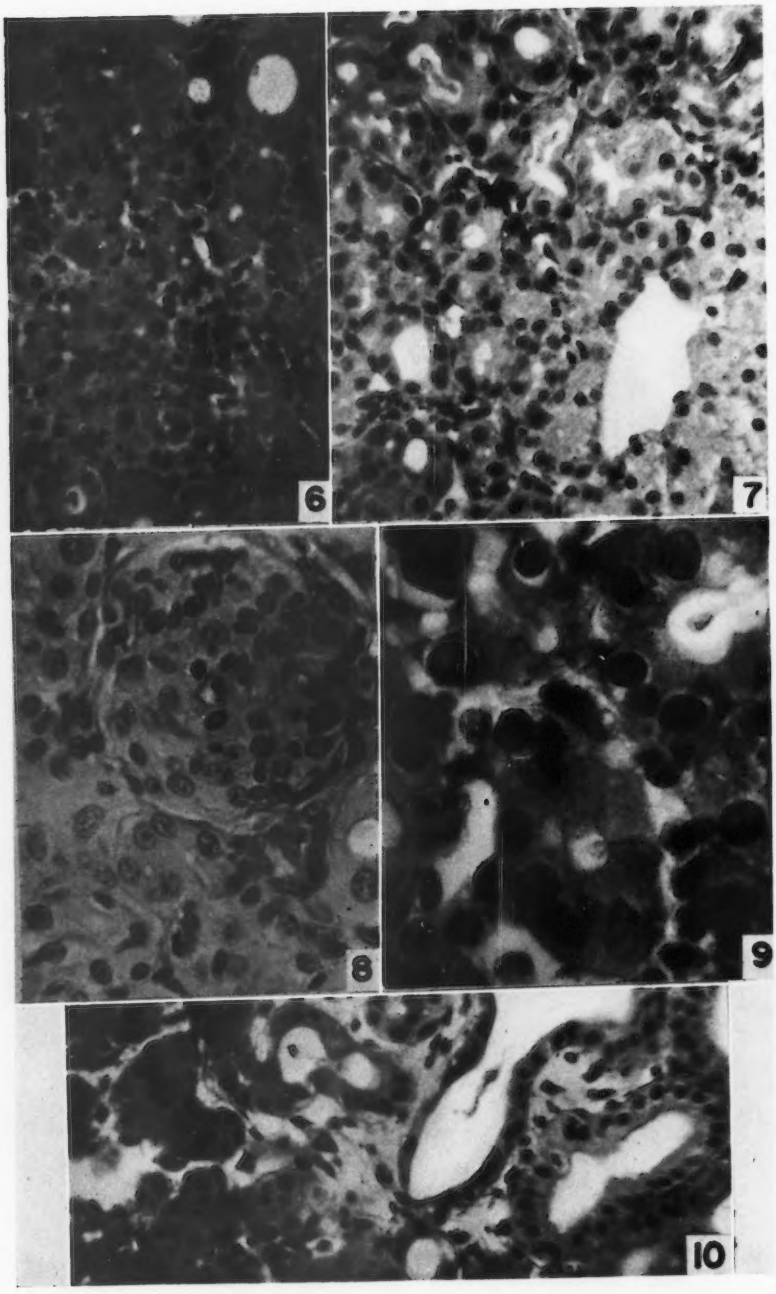
FIG. 3. Section of the thyroid gland of a tadpole exposed to 2-acetylaminofluorene solution for 43 days. $\times$230.

FIG. 4. Section of a normal frog tadpole liver. $\times$460.

FIG. 5. Section of the liver of a tadpole exposed to 2-acetylaminofluorene solution for 35 days. Note swollen cells and dilated bile capillaries. $\times$460.

PLATE I

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respectively while the average length of the control tadpoles was 53.75 ± 6.61 mm. The experimental animals had developed only as far as stages IV and VI while the control animals had developed as far as stages IX and X. Hind limb buds appeared in some of the treated animals but none survived through metamorphosis.

Effects of 2-aaf on Tail Regeneration

Two groups of 15 tadpoles each were selected, all having the same total length and having developed from the same egg mass. One group was immersed in a solution of 2-acetylaminofluorene (1.3 mgm. per 100 cc.) and the other group was maintained in tap water. After 48 hr. the distal one-third of the tail was amputated. The experimental animals were raised in 2-acetylaminofluorene solution and the control animals in tap water. The length of the regenerate in each case was measured daily. When regeneration was complete in the control group the tails of both groups of animals were amputated a second time at a level immediately anterior to the previous level of amputation. Again the length of the regenerates was measured from time to time.

During the first regeneration the difference in rate of regeneration in the two groups was slight, and on the 17th day after amputation of the tail there was no significant difference between the average length of the regenerates in the two groups. The difference in rate of tail regeneration became marked during the second regeneration. By the 17th day following the second amputation 6 of the original 15 animals in the experimental group had survived. The average length of the regenerated portion of the tails in the control group was 7.91 ± 0.073 mm., while that of the treated group was 5.71 ± 0.02 mm. This difference is significant at the 0.005 level.

Effects of 2-aaf on Melanophores

All of the finger bowls containing tadpoles were usually kept on light colored towelling paper in a well lighted place by a window, but not in direct sunlight. The control animals were, therefore, usually blanched. Tadpoles maintained under these conditions in 2-acetylaminofluorene solutions became very dark after 24 hr. exposure and remained dark thereafter. When these animals were examined under a dissecting microscope both the melanophores of the skin and also the perivisceral melanophores were seen to be completely expanded.

FIG. 6. Section of the liver of a tadpole exposed to 2-acetylaminofluorene solution for 44 days. ×230.

FIG. 7. Section of the liver of a tadpole exposed to 2-acetylaminofluorene solution for 46 days. ×230.

FIG. 8. Section of the liver of a tadpole exposed to 2-acetylaminofluorene solution for 47 days. Note the nodule of epithelioid cells at upper right. ×460.

FIG. 9. Section of the liver of a tadpole exposed to 2-acetylaminofluorene solution for 63 days. ×740.

FIG. 10. Section of the liver of a tadpole raised in 2-acetylaminofluorene solution for 53 days and in tap water for 14 days. Note proliferation of bile ducts and development of connective tissue. ×460.

Microscopic examination of the thyroid glands of eight control and eight treated animals up to 43 days' exposure to the carcinogen revealed no discernible alterations in the experimental glands (Figs. 2 and 3). With regard to size of gland, amount of colloid present, number of follicles, height of epithelial cells, etc., the thyroid glands of control and experimental animals were indistinguishable.

Effects of 2-aaf on the Liver of the Frog Tadpole

Frog tadpoles were maintained as long as possible in solutions of 2-acetylaminofluorene. When animals died or were sacrificed they were dissected under a dissecting microscope. No gross changes were observed in any organ other than the liver. Microscopic examination of the kidneys revealed no appreciable alterations in structure. The liver was both grossly and microscopically affected by the test substance.

The liver of the frog tadpole (Fig. 4) is not subdivided into lobules as it is in the mammal. The tadpole liver is a much branched tubular structure, the terminal branches of which enclose the ultimate ramifications of the hepatic ducts, the bile capillaries. The bile capillaries branch and anastomose in an irregular manner and thus obscure the original tubular structure of the organ. Seen in cross section the bile capillaries are surrounded by two or three, rarely more than four, cells. The secreting cells of the liver are large, cuboidal or polyhedral in form, with large conspicuous nuclei. The cytoplasm of these cells is coarsely granular. Even in large actively growing tadpoles mitotic figures are remarkably scarce in the parenchymal cells. In the normal tadpole liver the connective tissue framework is sparse.

After 35 days of treatment in 2-acetylaminofluorene solution (1.3 mgm. per 100 cc.), the liver (six tadpoles) was normal in shape and size but darker in color. The gall bladder was very much distended. There were scattered groups of enlarged hepatic cells with very large nuclei and distinct nucleoli. The cytoplasm of these cells was nonvacuolated and more homogeneous than that of normal neighboring cells. In some areas the bile capillaries were somewhat dilated (Fig. 5). Mitotic activity in these cells was increased. There were small areas of degeneration in some sections. The liver of another tadpole, treated for 36 days, presented the same appearance.

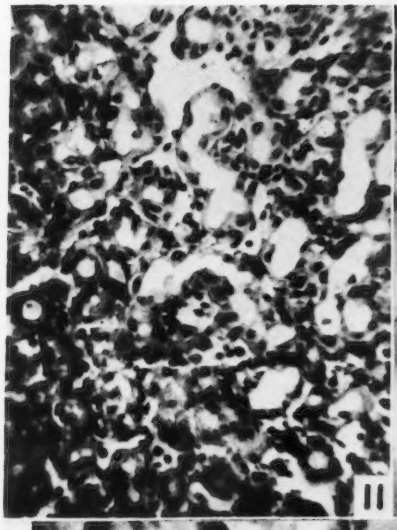
After 44 days of treatment (1.3 mgm. per 100 cc. solution) (four tadpoles) these changes in the liver had become more marked. Swollen, smooth cells, with enlarged pale nuclei and prominent nucleoli, and dilated capillaries were

FIG. 11. Section of the liver of a frog tadpole raised in 2-acetylaminofluorene solution for 53 days and in tap water for 21 days. Dilation of the bile capillaries with increase in cells presents the appearance of an adenoma. ×180.

FIG. 12. Section of the liver of a tadpole exposed to a 0.65 mgm. per 100 cc. solution of 2-acetylaminofluorene for 69 days. Note enlarged cells and nuclei. ×740.

FIG. 13. Section of the liver of a tadpole exposed to a 0.65 mgm. per 100 cc. solution of 2-acetylaminofluorene for 114 days. On the left is a mass of indistinct cells having a trabecular appearance. ×460.

FIG. 14. Section of the liver of a tadpole exposed to a 0.65 mgm. per 100 cc. solution of 2-acetylaminofluorene for 127 days. ×230.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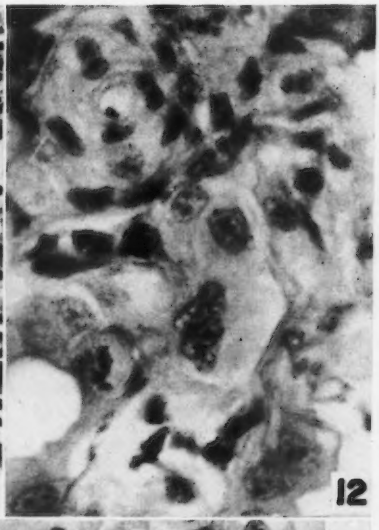
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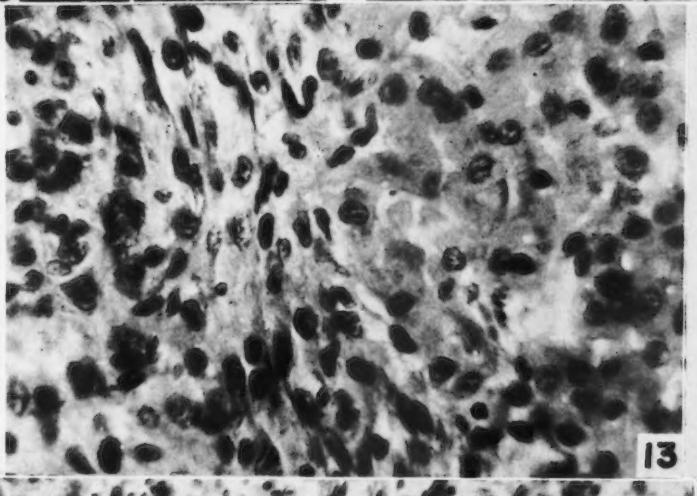
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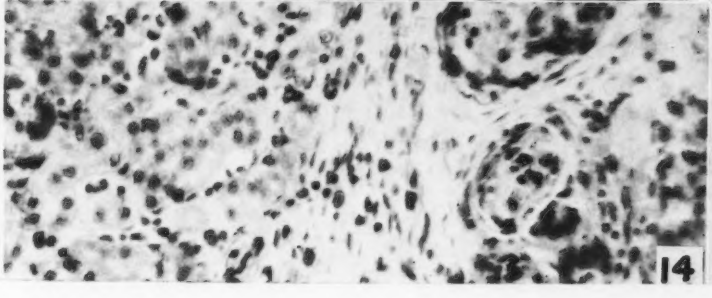
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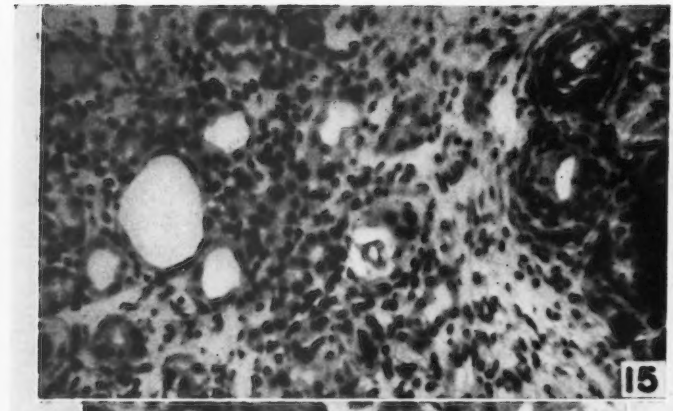
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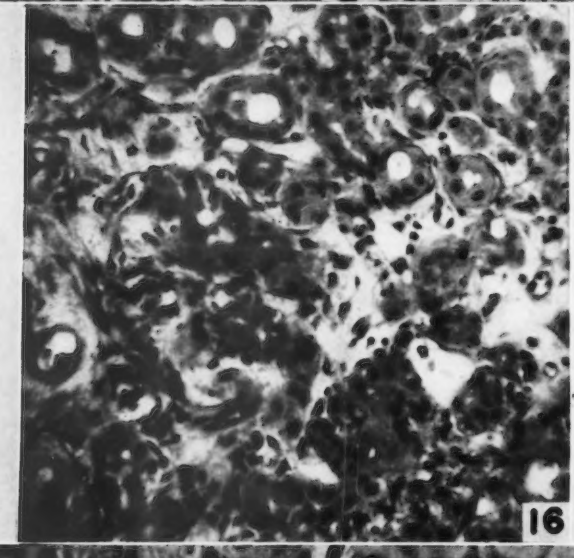
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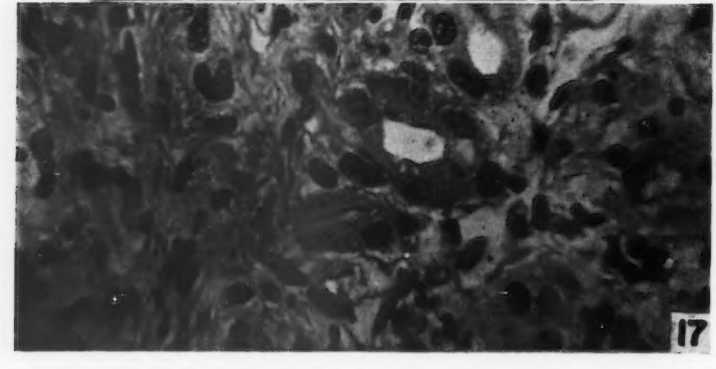
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more numerous (Fig. 6), and there was some proliferation of the bile ducts. In two tadpoles similarly treated for 46 days the distortion of the liver structure was more distinct. There were pale yellowish areas on the surface of the liver. The bile capillaries, which were enlarged and surrounded by flattened liver cells (many of which were dividing or were multinucleate) presented the appearance of irregular tubes (Fig. 7). There was also some proliferation of the bile ducts. The surfaces of the livers of three tadpoles raised in the same concentration of the carcinogen for 47 days were covered with many minute nodules. The microscopic appearance of these livers was similar to that already described except that scattered throughout the sections were small nodular areas composed of epithelioid cells whose cell boundaries were not easily distinguishable (Fig. 8). The nuclei of these cells varied in size and were irregular in outline. There were occasionally some mitotic figures at the outer margins of these masses but rarely within them.

Four tadpoles were sacrificed after 51 days' exposure to the 2-acetylaminofluorene solution (1.3 mgm. per 100 cc.). The livers of these animals differed very little in microscopic appearance from those previously described. The dilation of the bile capillaries with increase in the number of cells surrounding them was marked. After 63 days of treatment (three tadpoles) the number of widely dilated bile capillaries had become more numerous, giving the liver sections an adenomatous appearance. The flattened liver cells surrounding the bile capillaries contained several small nuclei or one very large nucleus (Fig. 9). There were some small areas of cells with indistinct cell boundaries; many of the cells were dividing.

Two tadpoles, treated with the 2-acetylaminofluorene solution (1.3 mgm. per 100 cc.) for 53 days, were transferred to tap water for 14 days. The surface of the liver of each of these animals showed a number of pale yellowish areas. Microscopically some areas of the sections presented the same adenomatous appearance as that just described. Proliferation of the bile ducts and a sharp increase in connective tissue were also apparent (Fig. 10). The livers of two other animals maintained in tap water for 40 days following 56 days' exposure to the 2-acetylaminofluorene solution appeared essentially the same, with the exception that small nodules of concentric epithelioid cells (as in Fig. 8) were observed in the sections.

In three tadpoles, treated with the solution of the carcinogen (1.3 mgm. per 100 cc.) for 53 days and subsequently maintained in tap water for 21 days, the livers were deformed and enlarged, with extensive pale yellowish areas on the surface. Microscopic examination of these livers showed extensive areas of degeneration. Mitotic activity was relatively great. Dilated bile

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FIG. 15. Section of the liver of a tadpole exposed to a 0.65 mgm. per 100 cc. solution of 2-acetylaminofluorene for 133 days. ×180.

FIG. 16. Section of the liver of a tadpole under the same conditions as in Fig. 15. Note adenomatous appearance of the liver. ×230.

FIG. 17. Section of a graft from a tadpole raised in 2-acetylaminofluorene solution for 114 days and recovered from the host tadpole after eight days. ×460.

capillaries gave these sections an adenomatous appearance (Fig. 11). There was also an increase of connective tissue.

One tadpole was sacrificed after 69 days' exposure to a 0.65 mgm. per 100 cc. solution of 2-acetylaminofluorene. The liver was very much enlarged and deformed. Microscopically, the normal architecture of the liver was almost completely altered. There were many nodular areas of concentrically arranged epithelioid cells (Fig. 12) whose nuclei were irregular in shape and varied in size. Some of these cells appeared to be dividing amitotically, but mitotic figures were confined to the outer margins of the nodules. There were occasional giant cells with very large nuclei. Masses of cells with indistinct cell boundaries were also present.

After 114 days of exposure to the same concentration of the carcinogen the liver of one tadpole had become more than three times normal size. It caused the body wall to protrude ventrally and laterally. The viscera were displaced but not invaded by it. The liver was hard and ivory white in color. The hepatic vessels present on the ventral surface of the normal liver were not distinguishable. The normal architecture of the liver was completely abolished and few normal liver cell cords remained. There were many nodular areas with the same characteristics as those already described. Extensive connective tissue made up a large part of the liver. Mitotic figures were abundant in connective tissue cells and liver cells. There were several areas of cells not forming normal liver cords, but running together with indistinct cell boundaries and irregular nuclei, and presenting a trabecular appearance (Fig. 13). These masses had the appearance of streams of cells infiltrating normal liver cell arrangements. Three tadpoles, sacrificed after 121 days', 127 days', and 133 days' exposure in 0.65 mgm. per 100 cc. solution of the carcinogen presented essentially the same features as those described above (Figs. 14 and 15).

Four tadpoles survived for 122 days in a 1.3 mgm. per 100 cc. solution of the carcinogen. The liver was enlarged in all cases and in one case it almost filled the body cavity, but had not invaded the other viscera. Essentially the microscopic appearance of these livers was the same as those described above (Fig. 16).

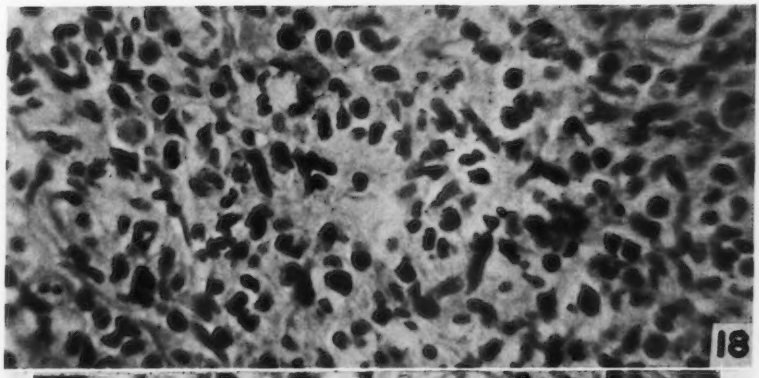
Fate of Liver Tissue Transferred from Treated Tadpoles to Control Tadpoles

The liver was dissected out of frog tadpoles previously treated with 2-acetylaminofluorene as described below. Fragments of liver tissse, 1–2 mm. in diameter, were immediately inserted into the body cavity of normal tadpoles.

FIG. 18. Section of a graft from a tadpole exposed to the carcinogen for 74 days and recovered from the host tadpole after 22 days. ×460.

FIG. 19. Section of a graft from a tadpole treated with the carcinogen for 74 days and recovered from the host tadpole after 27 days. Note adenomatous appearance of remaining liver cells. ×360.

FIG. 20. Section of a graft from a tadpole treated with the carcinogen for 114 days and recovered from the host tadpole after 47 days. The graft seems to have invaded the body wall of the host. ×230.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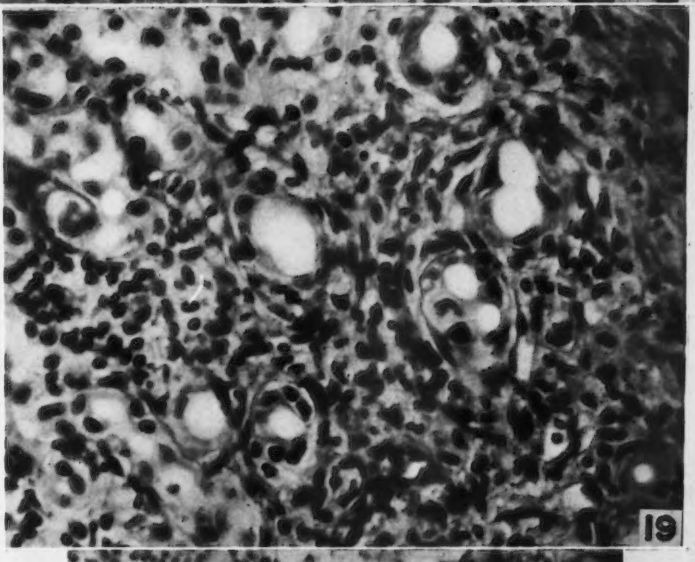
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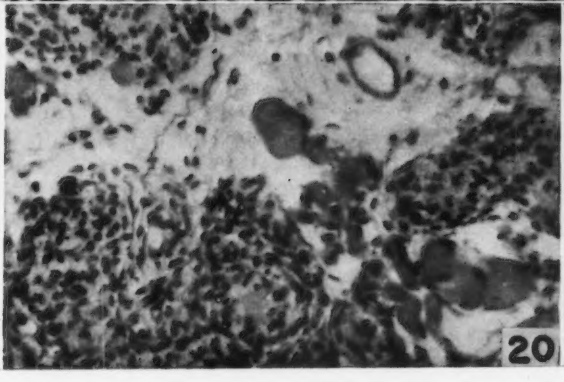
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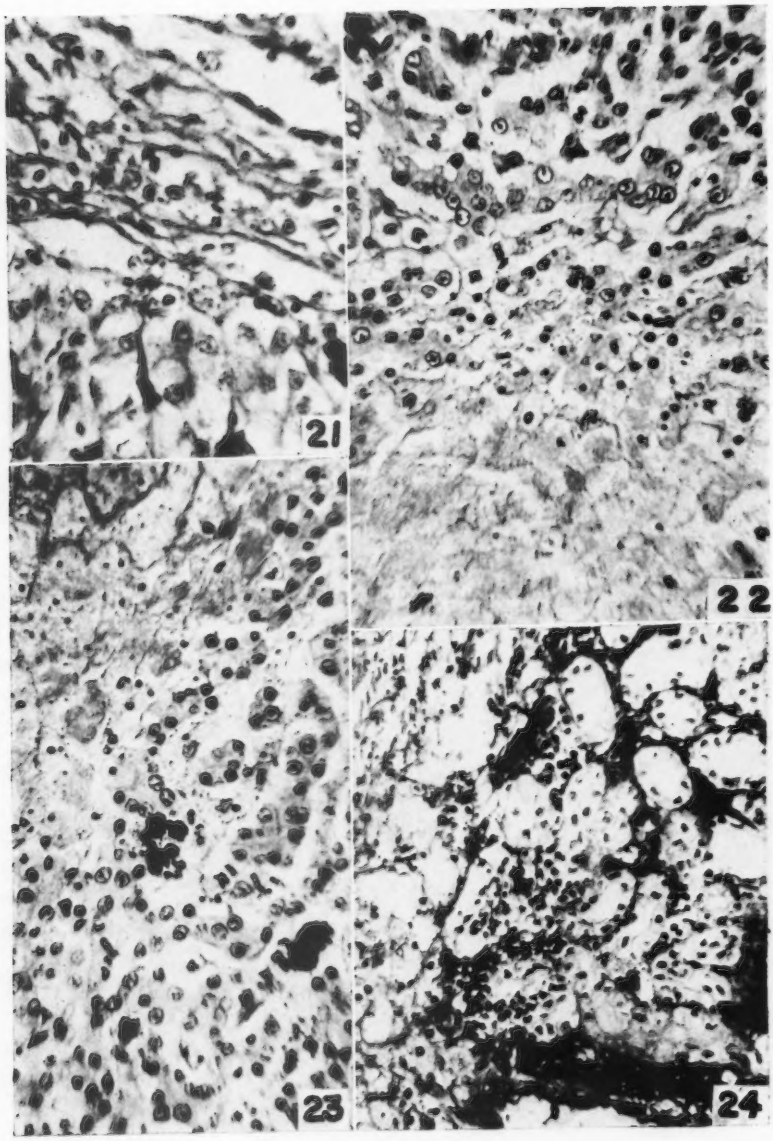
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In general these grafts did not survive beyond 30 days, but became necrotic and were resorbed. In seven cases the grafts were viable when recovered and showed some mitotic activity. Growth of the grafts, however, was negligible, and serial transplants were not possible.

Liver tissue inserted into the body cavity of normal tadpoles from tadpoles exposed for 36 days and 42 days to 2-acetylaminofluorene solution (1.3 mgm. per 100 cc.) was resorbed quickly. One piece of tissue, recovered after 20 days, still contained a few cells recognizable both in structure and arrangement as liver cells. The tissue was well vascularized, but composed chiefly of connective tissue. A similar piece of tissue, recovered after 30 days, was also composed chiefly of connective tissue. Pieces of the liver of a tadpole, exposed to the same solution of the carcinogen for 64 days and inserted into the body cavity of normal tadpoles, were recovered 13, 16, and 27 days later. These were all necrotic masses surrounded by a layer of connective tissue and were very small.

Pieces of the liver of a tadpole treated with the carcinogen (1.3 mgm. per 100 cc.) for 74 days were inserted into the body cavity of normal tadpoles. Two pieces were recovered after 22 days, one after 32 days, and two after 37 days. Both of the fragments recovered after 22 days were well vascularized and securely attached to the body wall of the host by connective tissue. There was no evidence of necrosis in either fragment. Both consisted of a more or less solid mass of epithelial cells no longer recognizable as liver cells (Fig. 18). The piece of liver tissue recovered after 27 days consisted of two parts which were well vascularized and securely attached to the body wall of the host. One part was similar to those just described. The other part (Fig. 19) presented the appearance of an adenoma. These was no evidence of any invasive action of the tissue. After 32 days and 37 days the liver tissue was necrotic and almost completely resorbed.

Pieces of the liver of a tadpole raised for 114 days in a 0.65 mgm. per 100 cc. solution of 2-acetylaminofluorene were inserted into the body cavity of normal tadpoles and subsequently recovered for study. At eight days the tissue had not become vascularized and was becoming necrotic. The surviving tissue was largely connective tissue but some liver cells, some of which were dividing mitotically, were still present (Fig. 17). A piece of tissue recovered after 19 days was almost completely resorbed. A graft recovered after 31 days was well vascularized. Scarcely any tissue recognizable to liver tissue was present, but several areas of concentric epithelioid cells persisted. Connective tissue made up the greater part of the remaining healthy tissue. A

FIG. 21. Section of the liver of an adult frog fed 2-acetylaminofluorene for 53 days. ×320.

FIG. 22. Section of the liver of a frog fed 2-acetylaminofluorene for two months. Note large area of necrosis. ×210.

FIG. 23. Section of the liver of a frog fed 2-acetylaminofluorene for 53 days. Compare with Fig. 22. ×210.

FIG. 24. Section of the kidney of a frog fed 2-acetylaminofluorene for 53 days. Note large area of necrosis at upper left. ×120

piece of tissue recovered after 41 days appeared to have survived more successfully. There was considerable mitotic activity in this tissue, and some elongated nuclei appeared to be dividing amitotically. There were both nodular areas and areas of cells with indistinct cell boundaries. Connective tissue was extensive. A graft of liver tissue, recovered after 47 days, had the same general appearance, but seemed to have invaded the muscles of the body wall of the host (Fig. 20).

Effects of 2-aaf on Adult Frogs

Several methods of treating adult frogs with 2-acetylaminofluorene and 2-aminofluorene were tried. These substances were highly toxic to the frogs, even in very small amounts. One frog survived for two months, during which time it had been fed a total of 10 mgm. of 2-acetylaminofluorene as crystals in gelatin capsules at weekly intervals. Two frogs survived for 53 days, during which time each had been fed a total of 8.25 mgm. of the carcinogen in olive oil in gelatin capsules on alternate days. Each capsule contained 0.25 mgm. of 2-acetylaminofluorene in 0.1 cc. olive oil. In one of these frogs there was some proliferation of connective tissue in the liver (Fig. 21). In all three cases there was extensive liver damage, indicated by small areas of necrosis throughout the liver (Figs. 22 and 23). Hyperchromatic or pycnotic nuclei and degenerating cells with signet ring nuclei were present. There was some mitotic activity in the liver sections, suggesting a compensatory regeneration. There was also a massive area of necrosis in the kidney of one of these animals (Fig. 24).

Discussion

In comparison with the vast amount of work that has been carried out in the study of neoplasia and carcinogenesis in mammals the amount devoted to a study of these phenomena in the Amphibia has been relatively little. Attempts to induce neoplasia in the Amphibia with chemical carcinogens have been largely unsuccessful. As a result it has been often stated that the Amphibia are less disposed to tumor formation than are other classes of vertebrates, or that they are refractive to carcinogens (21, 54, and other references).

Wilson (57) discovered that 2-acetylaminofluorene was a powerful carcinogen. It produced carcinoma of the liver, lung, bladder, pancreas, and renal pelvis in rats. These findings have been subsequently confirmed by Bielschowsky (8, 9) and by many others. This carcinogen has a wide range of activity, having induced tumors in all of five species tested: rat (57); mouse (5, 6); fowl (13); cat (11); and the dog (4). The most frequently occurring tumors are benign and malignant neoplasms of the liver (4, 19, 20, and other references).

Even in large doses (50 mgm. per kgm. of body weight) 2-acetylaminofluorene did not produce any acute toxic reactions in rats, mice, or rabbits (57). It has also been shown that as little as 0.004% of 2-acetylaminofluorene in the diet for 25 days produces changes which later become cancerous (58, 59).

The important feature of the effect of 2-acetylaminofluorene on the development of the frog embryo is its irregularity. The effects of this carcinogen are somewhat similar to those described by Briggs and Briggs (18) resulting from exposure to water soluble derivatives of dibenzanthracene and methylcholanthrene. Briggs and Briggs also reared frog embryos in solutions of related noncarcinogenic substances and have shown that the results that they obtained were specific for the carcinogens. From a comparison of their results with the results of the present study, it would seem that the retardation of the rate of development in postgastrula stages by 2-acetylaminofluorene is similar to retardation caused by other carcinogenic solutions. However, most of the effects produced in this study (development of exogastrulae, hemiembryos, etc.) are similar to those resulting from treatment with a number of toxic substances (50).

The growth inhibiting character of many carcinogenic hydrocarbons has been extensively studied by Haddow and his associates (28, 29, 30, 31, 32). Haddow and Robinson have shown that there is a close statistical association of the biological properties of carcinogenicity and growth inhibiting powers. These authors have suggested that these substances exert their effects by way of the pituitary gland. In agreement with their findings, it has been shown in the present study that 2-acetylaminofluorene inhibited the rate of growth and development of frog tadpoles, and also the rate of regeneration of the tail in these animals. Similar observations have been reported by previous investigators. Bielschowsky and Greene (12) found that both 2-acetylaminofluorene and 2-aminofluorene inhibited the growth of bacteria and rats. Wilson, DeEds, and Cox (57) and Engel and Copeland (23) have shown that rats to which these substances were fed failed to grow. Carcinogenic substances have been shown to inhibit regeneration in the urodeles (48, 55).

The mode of action of carcinogenic substances in the inhibition of regeneration is not well understood. It is possible that, as suggested by Haddow and Robinson (31) for other substances, fluorene derivatives exert their growth inhibiting action by way of the pituitary gland. The pituitary gland controls the growth of frog tadpoles (2, 3) as in other animals. Removal of the pituitary body inhibits growth and regeneration. Herrell (33) stimulated the rate and amount of regeneration by injections of pituitary extract, while Puckett (47) found that such injections enhanced the growth of frog tadpoles but did not appreciably increase the rate of regeneration. Contrary to what might be expected from the results of studies on mammals (9, 10, 27, 37), 2-acetylaminofluorene apparently had no effect upon the thyroid gland of the tadpoles, at least for the time of exposure studied. Furthermore, although inactivity of the thyroid gland inhibits regeneration (24) the same condition produces giant tadpoles that fail to metamorphose (26).

The effects of the carcinogen upon the melanophores of the frog tadpole are apparently not secondary to inhibition of the thyroid gland, because inhibition of the thyroid gland causes "blanching" (14, 36, 41). On the other hand, contraction and expansion of the melanophores is controlled chiefly by

the intermediate lobe of the pituitary body (1, 7, 25, 34, 35). Absence of the pituitary body results in a silvery condition, while extracts of it cause dilation of the melanophores. The most probable explanation of these two opposing effects, inhibition of growth and dilation of the melanophores, is that they are unrelated phenomena. Growth inhibition may be effected through the pituitary gland. The dilation of the melanophores may be either a local action of the drug of an anesthetic nature or possibly a protective response to a photochemical synergism.

The effects of 2-acetylaminofluorene on the liver of the adult frog may be due only to the toxic action of this substance, but lesions of the liver of the tadpole were produced which are similar in many respects to those produced in the liver of the mammal by the same substance (19, 57). Under various conditions neoplastic lesions of the liver have appeared as early as 160 days after the beginning of treatment (19), and were similar to those induced by other carcinogens (22, 43, 44, 45). Hepatomas resulting from treatment with 2-acetylaminofluorene conformed to those described by Opie (43) as "adenohepatoma" and "trabecular hepatoma". In the adenohepatoma the cells were arranged in acinar formation, gradually merging into normal liver tissue. The cells of the trabecular hepatoma were large and polyhedral, often with irregularly shaped nuclei. These cells were frequently fused together or formed giant cells with multiple or lobulated deeply staining nuclei. The cell outlines were frequently indistinct and the cells appeared to be infiltrating between normal liver cell cords.

Dunning *et al.* (20) found that, in rats, lesions of the liver induced by 2-acetylaminofluorene included cirrhosis, hyperplasia of liver cells, and benign and malignant neoplasms. Similar lesions have been reported in the liver of the dog (4). The morphology of these tumors was similar to that of lesions obtained by Opie (43) with butter yellow. Dunning *et al.* found cirrhosis in 35 of 50 rats; 80% in three strains; 100% in a fourth strain; and 10% in a fifth strain. Benign hepatomas were all associated with cirrhosis. Opie pointed out that whether cirrhosis is or is not a necessary preliminary in carcinogenesis, there appears to be no doubt that the yield of tumors is greater when it occurs. Most pathologists seem to believe that cirrhosis precedes and influences the development of liver cell carcinoma (38, 42).

In the liver of the frog tadpole exposed to solutions of 2-acetylaminofluorene the formation of connective tissue, comparable to cirrhosis in the mammal, became evident as early as 69 days, and was extensive. The increase in connective tissue was accompanied by tissues having histologic and morphologic resemblance to neoplastic tissues described as "adenohepatoma" and "trabecular hepatoma" by several authors (19, 43).

Although the changes observed in the liver of the frog tadpole following exposure to 2-acetylaminofluorene are strikingly like those described in mammals, there is no certain evidence that they are definitely neoplastic, either benign or malignant. They may, however, at least be regarded as lesions of the type that precede or accompany the development of hepatomas

in mammals. Whether any of the conditions described in the frog tadpole might go on to develop definitive tumors is also uncertain. The evaluation of the presence or absence of a tumor of the liver is uncertain in the mammal, which has been extensively studied (22). In mammalian experiments it is often questionable whether a tumor of the liver exists or whether any given lesion is or is not neoplastic. Many authors (15, 43) have stressed the fact that histological structure is an uncertain index of malignancy.

The evidence gained from transplantation of liver tissue from animals exposed to 2-acetylaminofluorene to normal tadpoles is not entirely satisfactory. Although there was evidence of initial growth most of the transplanted tissues were necrotic and partly resorbed when recovered. One of the grafts (Fig. 18) was composed of healthy tissue having no resemblance to liver tissue, a second (Fig. 19) presented an adenomatous appearance, and a third (Fig. 20) appeared to be invading the muscles of the body wall of the host. Transplants of the Lucké kidney carcinoma are successful only on an average of 50% of the time and frequently as little as 10% (40, 51). There is some doubt that, with the exception of this tumor and a melanoma of axolotl (52), any suspected tumor of the Amphibia has been successfully transplanted, although Breedis (16) has recently reported a transplantable sarcoma of the salamander induced by methylcholanthrene.

Acknowledgment

The author is indebted to Prof. J. Walter Wilson of Brown University for his interest in this investigation and for his many helpful criticisms.

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SIAMESE TWIN EMBRYOS OF THE BLACK WIDOW SPIDER[1], *LATRODECTUS MACTANS* (FABR.)

By J. G. Rempel[2]

Abstract

Two sets of Siamese twin embryos of the black widow spider are described. The first set is 290 hr. old, the second, 333 hr. In both cases duplication involves the cephalothorax. A description of the internal structures based on normal embryos of the same age is given. The opinion is expressed that such twins could not survive beyond blastokinesis.

Studies of anomalies of spiders seem to have been limited to studies of gynandromorphism. Exline (1) gives a detailed description of the external and internal characteristics of four gynandromorphs of American spiders and reviews the literature on the subject. A search through published works, however, has not revealed studies of twinning in spiders.

In the summer of 1952, while studying the embryology of the black widow spider,[3] the writer (2) discovered two Siamese twin embryos. Since few investigations of anomalies of spiders have been published, it is felt that a description of these is in order.

Adult female spiders collected at Govenlock in southern Saskatchewan on July 4, 1952, were taken to the laboratory and kept alive until late fall. Of 14 spiders, 12 oviposited in captivity, some producing as many as three egg cases. The eggs were kept at room temperature until fixation. Spider No. 11, at the time of collecting, was guarding two egg cases. On July 18 a third egg sac was produced, with some one hundred and fifty eggs. Two of these eggs developed into Siamese twins. Since discovery of this was made after fixation, we do not know whether the individuals could have survived blastokinesis.

Twins, First Set (*Fig. 1*)

This is a 290-hr. stage. The yolk has been removed to show the embryo clearly. The duplication involves the cephalothorax only. The thoracic (*leg*) and cephalic (*pdp chl*) appendages, with the exception of the rostral, are evident. They all present the same appearance and are directed caudad. The abdomen has undergone segmentation and has six segments, of a definitive number of eight. Abdominal appendages have not yet appeared. Sections of normal embryos of the same age disclose two body layers, ectoderm and mesoderm. Fig. 2 is a reconstruction of this specimen cut in a horizontal plane. The mesoderm (*mes*) is segmented and in the cephalothorax is restricted to the appendages (*leg*). In the abdomen the somites have split along the midventral line and have formed the coelomic sacs (*coel*). The ectoderm (*ect*) is of uniform thickness and there is no indication that

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Contribution from the Department of Biology, University of Saskatchewan, Saskatoon, Sask.
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[3] *This investigation was supported financially by the National Research Council of Canada*

blastokinesis has set in. In the extraembryonic area (*ext*) the yolk is largely uncovered, except for a few spindle-shaped cells which are regarded as extensions of the ectodermal layer. Vitellophags (*yc*) have broken away from the ectoderm and have penetrated the yolk (*y*).

Twins, Second Set (*Figs. 3, 4, 5, 6*)

This is a 333-hr. stage. As in the previous case, duplication involves the cephalothorax only (Fig. 3). Development, however, is more advanced than in the former. The rostral appendages have appeared and fused to form what Snodgrass (3) calls the labrum (*lm*). Immediately behind the labrum is an ectodermal invagination, the stomodaeum (*stom*). The chelicerae (*chl*) have rotated through 90° and are now directed toward the mid-ventral line of the embryo. The pedipalps (*pdp*) have undergone segmentation and the coxal lobes have appeared; a rotation similar to that of the chelicerae has occurred. The thoracic appendages (Fig. 4, *leg*) are long and narrow and appear to be four-segmented. These, too, have rotated through 90°. Segmentation of the abdomen is complete, with the definitive number of eight segments (*ab*) and a telson (Fig. 3, *tel*). Abdominal appendages have appeared on the second to the fifth segments.

Blastokinesis in this species is similar to that of many other spiders. The ectoderm becomes thin along the mid-ventral line to form the ventral sulcus (Fig. 4, *sulv*). The latter extends from the labrum to the telson and its widening is responsible for the lateral expansion of the body to cover the extraembryonic area (*ext*). The arrows in Figs. 3 to 6 indicate the direction of the expansion. As the cells in the ventral sulcus become stretched and flattened, the region becomes transparent and yolk spheres can be seen beneath the ectoderm (Fig. 4). Coincident with the appearance of the ventral sulcus, the first traces of the central nervous system become evident. On either side of the ventral sulcus the ectoderm thickens to form ganglionic masses. The cheliceral ganglia (*gchl*), the ganglia of the pedipalps (*gpdp*), and the abdominal ganglia (*gab*) are seen in Fig. 3. In the head the large cephalic lobes (*ceph*) are evident on either side of the labrum. A reconstruction of a horizontal section of the stage shown in Fig. 3, and based on normal embryos of the same age, is shown in Fig. 5. Similarly, a reconstruction of a vertical section along the line *Y* (Figs. 3, 4) is shown in Fig. 6.

It is interesting to speculate whether Siamese twins of the black widow spider could survive. An answer to this question calls for an examination of the problem of blastokinesis in this species.

At approximately 310 hr. the normal embryo almost encircles the egg (Fig. 7). The extraembryonic areas at this time are extensive. It is the function of blastokinesis to cover the entire yolk mass with embryonic tissue in the manner described above. As the ventral sulcus appears and widens progressively (Figs. 8, 9), the lateral abdominal 'plates' spread over the yolk until they fuse with the edges of the cephalic region (Fig. 10). This accomplished, growth in the reverse direction sets in, resulting in a thickening of the ectoderm in the region of the ventral sulcus. In Siamese twin No. 2

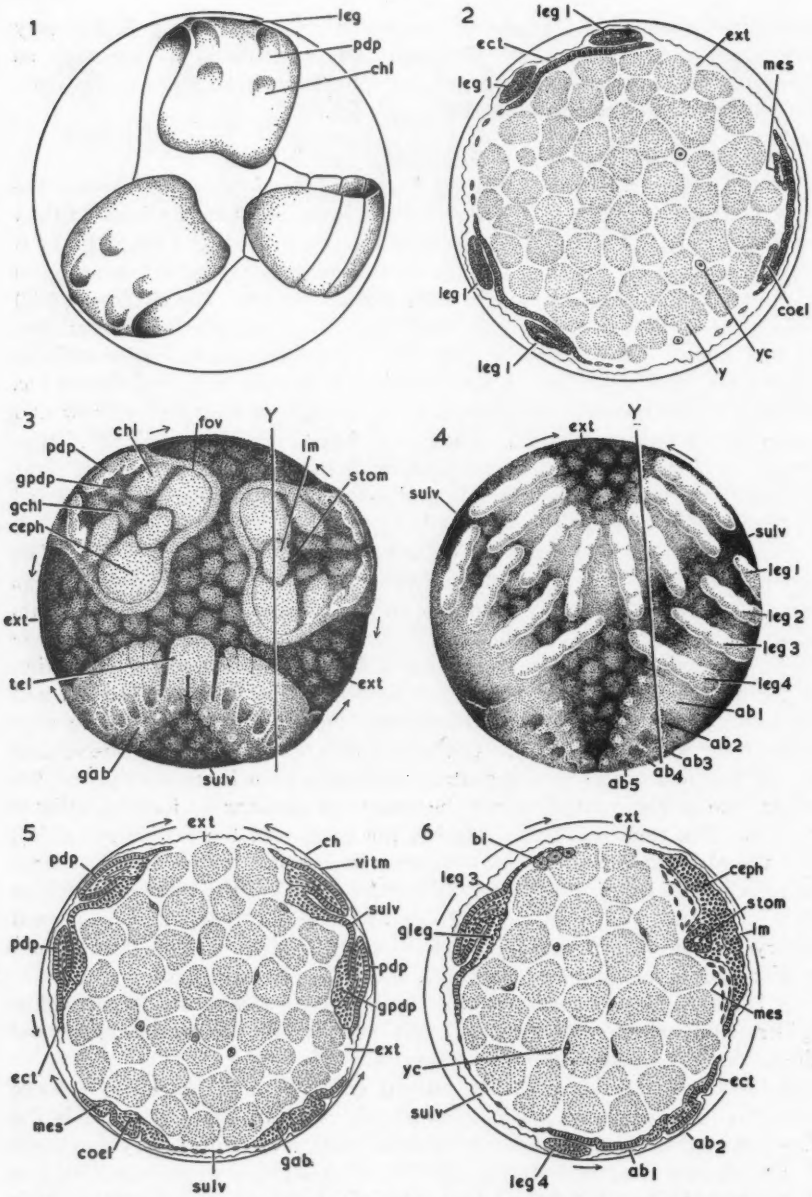

FIG. 1. Siamese twins, first set, 290 hr. old, dorsal view, with yolk removed. FIG. 2. Reconstruction of a horizontal section of above twins based on normal embryos of the same age. FIG. 3. Siamese twins, second set, 333 hr. old, dorsal view. FIG. 4. Siamese twins, second set, ventral view. FIG. 5. Reconstruction of horizontal section of second set, based on normal embryos of the same age. FIG. 6. Reconstruction of vertical section along line *Y* (Figs. 3, 4), based on normal embryos of the same age.

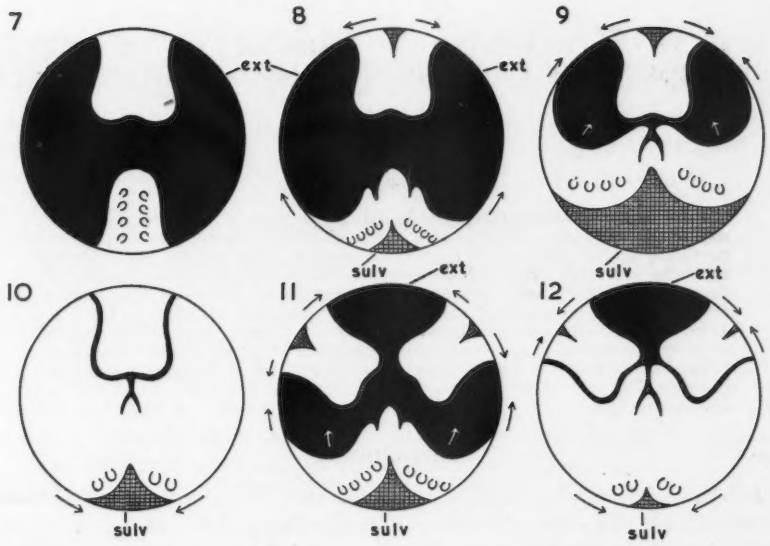

FIG. 7. Diagram of 310-hr. normal embryo, dorsal view. FIG. 8. Diagram of 330-hr. embryo in early stage of blastokinesis. FIG. 9. Diagram of 340-hr. embryo, at mid-point of blastokinesis. FIG. 10. Diagram of 350-hr. embryo, blastokinesis complete. FIG. 11. Diagram of Siamese twins, second set. FIG. 12. Diagram of hypothetical Siamese twins, blastokinesis complete.

(Figs. 3, 11), blastokinesis has set in. If we are justified in speculating further on a basis of our studies of normal embryos, the culmination of blastokinesis may be visualized as illustrated in Fig. 12. Since, under such circumstances, much of the extraembryonic area would be left uncovered permanently, the writer is inclined to believe that development of the twin embryo would be subverted to such an extent that it could not survive.

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EXPLANATION OF LETTERING ON FIGURES 1–12

ab	abdominal segment	*gab*	ganglion of abdominal segment	*stom*	stomodaeum
bl	blood	*gchl*	ganglion of chelicera	*sulv*	ventral sulcus
ceph	cephalic lobe	*gleg*	ganglion of leg	*tel*	telson
ch	chorion	*gpdp*	ganglion of pedipalp		
chl	chelicera	*leg*	leg	*vitm*	vitelline membrane
coel	coelom	*lm*	labrum		
ect	ectoderm	*mes*	mesoderm	*y*	yolk
ext	extraembryonic area	*pdp*	pedipalp	*yc*	vitellophag
fov	fovea				

EFFECTS OF THYROXINE AND THIOUREA ON THE EARLY DEVELOPMENT OF CHUM SALMON (*ONCORHYNCHUS KETA*)[1]

By Samuel Dales[2] and William S. Hoar[3]

Abstract

Eggs of chum salmon were incubated in solutions of synthetic thyroxine sodium or thiourea. Thyroxine accelerated growth of the body wall and pectoral fins but reduced the rate of increase in body length. In addition, thyroxine treatment produced exophthalmia, intense deposition of guanine, and decreased pigmentation. Thiourea likewise reduced the rate of growth in length but had no apparent effect on the development of body wall or fins. Decreased deposition of guanine was evident in thiourea treated fish but the deposition of melanin was not affected. Thyroids of fish treated with thyroxine showed characteristic colloid storage while those of fish in thiourea were hyperplastic. The rate of contraction of the embryonic heart was unaffected by the treatments.

Introduction

Available information indicates that the thyroid hormone of cold-blooded vertebrates is more directly concerned with growth and differentiation than it is with general metabolism. Contrary to the well known action of this hormone in mammals, thyroid preparations are usually reported to have no effect on the oxygen consumption of fish, amphibia, or reptiles. On the other hand, growth rate of fish, metamorphosis of amphibia, and molting in reptiles are all markedly influenced by thyroid hormone. Pertinent references will be found in current reviews (4, 5, 6, 9).

Several workers have described effects of thyroxine or various antithyroid compounds on growth and differentiation of young fish but the findings have not always been in agreement (5, 9, 10). In general, it can be said that there is still no clear picture of the function of thyroid hormone in differentiating tissues. The present investigation was therefore undertaken in an attempt to further an understanding of the role of this hormone in morphogenetic processes.

Although several investigators have studied the effects of thyroid preparations on the growth and differentiation of larval or juvenile fish, there are very few comparable experiments based on embryonic material. Baumann and Pfister (1) incubated eggs of rainbow trout in solutions of thyroxine and described increased growth and a modified development of the aortic arches. Warner (11) incubated brown trout eggs in thiouracil and observed a slight delay in hatching time but no particular change in growth rate. Several recent experiments carried out by Russian workers are reviewed by Chambers (3). In the present study, eggs of chum salmon (*Oncorhynchus keta*) have

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Contribution from the Zoology Department, University of British Columbia, Vancouver, British Columbia. These data are taken from a thesis presented by S. Dales for the M.A. degree in Zoology and have been prepared for publication by W. S. Hoar.

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been incubated in thyroxine or thiourea. The conspicuous variations produced by this treatment were different from those described in trout. It may be of interest that the chum salmon has one of the largest of teleost eggs (diameter about 8.0 mm.). The prolonged larval development with extensive growth of the body wall required to cover the yolk mass has perhaps provided the most interesting features of the present study.

Materials and Methods

Eggs of chum salmon were obtained from fish migrating into Cultus Lake, B.C. The first series was collected on October 28, 1950; the second on November 10, 1951. In each case the eggs were fertilized at Cultus Lake and transported directly to the University (about four hours' journey) where they were divided into appropriate samples and placed in aquaria containing constantly aerated solutions. The aquaria stood in troughs of running water and the temperature, consequently, varied with that of the hatchery. During the course of experiments this ranged from about 10° C. to 13.0° C. in 1950 and from 5.5° C. to 12.0° C. in 1951. All solutions were changed weekly. Controls were maintained in similar numbers in aquaria containing tap water.

In the 1950 experiment, eggs were cultured in lots of 1000 and treated with synthetic thyroxine sodium (B.D.H.) in three different concentrations (1 : 2.5; 1 : 5.0; and 1 : 12.5 million) and thiourea in three different concentrations (0.05%, 0.1%; and 0.2%). After three months, eggs (embryos) treated with 0.1% thiourea were transferred to thyroxine (1 : 12,500,000). Three eggs were preserved daily in Bouin's picric acid – formol – acetic acid mixture.

In 1951 lots of 5000 eggs were treated with thiourea (0.05%) and with thyroxine sodium (1 : 12,500,000). Three eggs from each treatment were preserved on alternate days. At the end of the experiment (121 days after fertilization, 56 days after hatching) the remainder of the samples were preserved in Bouin's fixative. Two fish from each lot preserved at this time were embedded in paraffin, sectioned serially, and stained with haematoxylin and eosin. Additional procedures are given in connection with the results.

Results

Mortalities and Time of Hatching

Unfortunately control eggs for the 1950 experiment suffered a heavy attack of fungus 18 days after fertilization. Although the fungus was controlled by treatment with merthiolate, only 20 eggs hatched and the remainder died four days after the commencement of hatching. At this time a fresh sample of eggs was taken from the hatchery trough and used as "controls". Although fertilized on the same day, these hatched six days later than the original controls (57 days after hatching). This is not surprising, since the temperature of eggs cultured in aquaria was slightly higher owing to the constant aeration.

TABLE I

HATCHING TIMES IN CONTROL AND TREATED EGGS (1950)

Days after fertilization	Numbers hatched (cumulative)						
	Control	Thyroxine			Thiourea		
		1 : 2,500,000	1 : 5,000,000	1 : 12,500,000	0.05%	0.1%	0.2%
40	—	3*	2*	—	—	—	—
42	—	8*	10*	—	—	—	—
43	—	2*	4*	—	—	—	—
44	—	0	24*	25*	—	—	—
46	—	3*	40*	0	—	—	—
47	—	All dead	0	0	—	—	—
48	1	—	500*	6*	6	—	—
49	3	—	All dead	3	37	7	2
50	16	—	—	All dead	>500	35	20
51	20	—	—	—	—	>500	31
52	All dead	—	—	—	All hatched	—	300
53	—	—	—	—	—	—	—

* *Hatched dead.*

In spite of the unfortunate loss of the original controls it is evident from Table I that thyroxine, in proportion to its concentration, speeds up hatching time by four to eight days, whilst thiourea seems to have little effect. In the 1951 experiment hatching commenced at about 65 days in all groups with insignificant mortalities in both treated and control eggs. It is suggested that the unusually high 1950 temperatures in combination with thyroxine resulted in excessive acceleration of development and brought about almost complete hatching mortality. Hatching mortalities are frequently observed with adverse developmental conditions.

Relative Growth of Body Parts

General observation showed that fish which developed in thyroxine differed in a number of ways from the untreated controls. In the 1950 experiments, body length, appearance of eyes, and growth of body wall were studied. In 1951 effects on the growth of pectoral fins were particularly evident.

Body Length – Head Width

Mean body lengths of embryos from the 1950 experiments are shown in Table II. The difference between the mean length of control and thyroxine treated fish was statistically significant at the 99% probability level. As previous workers have found, thyroxine reduces the growth rate of developing fish. It will be noted that thiourea treated fish were likewise consistently shorter than the controls. Those treated 73 days were significantly shorter (99% probability level). Since the "controls" used for the 73 and 101 day measurements were from more slowly growing hatchery trough cultures, the difference is probably greater than the measurements indicate.

Thyroxine treated fish appeared to have wider skulls (Figs. 1 and 2). However, measurements of head width of samples of control and thyroxine

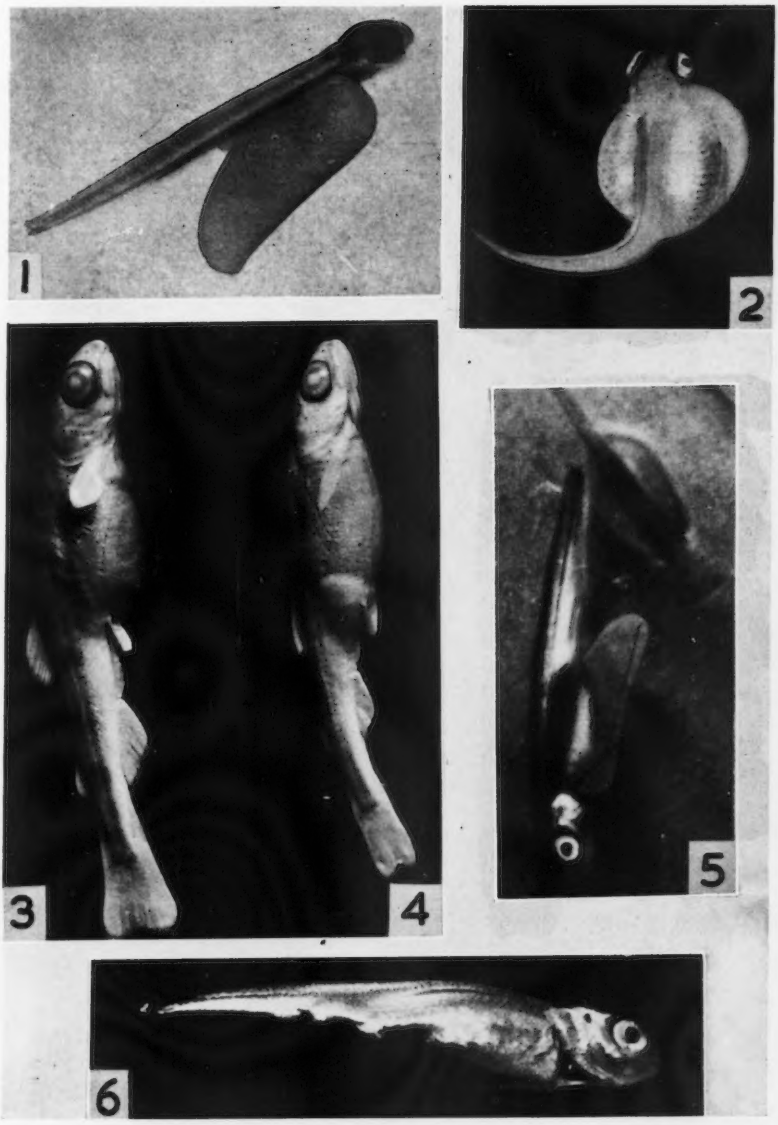

FIG. 1. Control embryo at time of hatching (1950 culture). FIG. 2. Thyroxine treated (1 : 12,500,000) embryo at time of hatching (1950 culture). FIG. 3. Control salmon, 121 days after fertilization, 56 days after hatching in 1951. FIG. 4. Thyroxine treated (1 : 12,500,000) salmon preserved 121 days after fertilization, 56 days after hatching in 1951. FIG. 5. Control fish 51 days after commencement of hatching in 1951. FIG. 6. Thyroxine treated fish, 51 days after commencement of hatching in 1951.

TABLE II

MEAN BODY LENGTHS FOR 20 FISH MEASURED FROM TIP OF SNOUT TO FORK IN TAIL (1950 CULTURE)

Days after fertilization	Means of body length in cm.				
	Control	Thyroxine 1 : 12,500,000	Thiourea 0.05%	Thiourea 0.1%	Thiourea 0.2%
48	2.39	1.89	—	—	—
52	2.29	—	2.22	2.19	2.11
73	2.77	—	2.55	2.56	—
101	3.17	—	2.95	2.99	3.28

treated fish did not differ significantly at the time of hatching. The apparent difference was probably due to a bulging of the eyes which appeared about 36 days after fertilization and developed into a marked exophthalmia at hatching time (Fig. 2). The optic cup formed at the same time in both control and treated fish (six to seven days after fertilization).

Body Length – Pectoral Fin Length

In 1951, approximately 70 fish were measured in each treatment group, for their body length and left pectoral fin length. Figs. 3 and 4 indicate that thyroxine treatment decreased body length and markedly increased pectoral fin length. The variations in length are of the same nature as those found in the 1950 experiments. Differences are shown graphically in Fig. 7. Body proportions were definitely altered by the treatments.

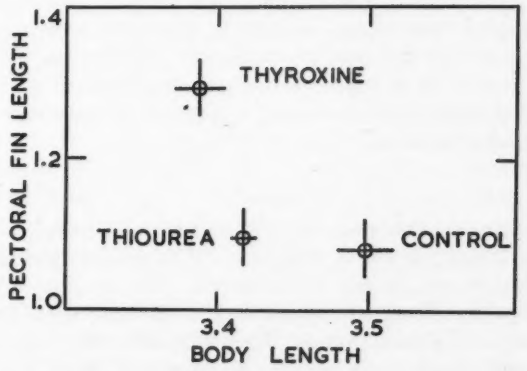

FIG. 7. Natural logarithms of mean lengths for body and pectoral fin. The vertical and horizontal lines give 99% fiducial limits for means of pectoral fin and body length respectively.

TABLE III

COMPARISON OF MEAN BODY LENGTH AND PECTORAL FIN LENGTH OF 70 CHUM ALEVINS 121 DAYS AFTER FERTILIZATION (1951 CULTURES)

	Body length, mm.			Pectoral fin length, mm.		
	Mean	Difference from control		Mean	Difference from control	
		Mean	*t*		Mean	*t*
Control	33.1	—	—	2.96	—	—
Thyroxine	29.7	3.4	13.6*	3.62	0.64	9.5*
Thiourea	30.6	2.5	10.0*	3.04	0.05	0.7

* *Significant difference. Tabled t at P .01 is 2.61.*

Growth of Body Wall

The body wall, consisting of layers of muscle and integument, begins to envelop the yolk sac several weeks after hatching, grows progressively downward until both sides join in the mid-line ventrally. In the 1950 control and thiourea treated alevins the body wall commenced to grow over the yolk sac about 60 days after fertilization. In the controls, the yolk sac was completely covered 99 days after fertilization while growth in the thiourea group became progressively retarded. The body wall began to cover the yolk sac 14 days before commencement of hatching in thyroxine treated eggs (26–33 days after fertilization), so that all embryos examined at hatching time showed some degree of downward growth (Fig. 2). In many instances, where the growth of the body wall had been particularly rapid, the pectoral fins were displaced downward. In the 1951 experiment, a precocious growth of body wall over yolk sac commenced some weeks after hatching and was photographed 51 days after fertilization (Figs. 5 and 6).

In 1950, alevins developing in 0.1% thiourea were transferred to 1 : 12,500,000 thyroxine solution, 84 days after fertilization. Within a short time the growth of body wall over yolk sac was accelerated to such an extent that within 12 days of commencement of thyroxine treatment the yolk sac had been covered completely.

Absorption of Yolk

In 1951, 25 fish were chosen at random from samples of fish fixed in Bouin's solution on the last day of the experiment (121 days after fertilization). Yolk sacs were dissected out, dried, and weighed. The weights for control and thyroxine and thiourea treated samples were 1.182 gm., 1.412 gm., and 1.313 gm. respectively. The rate of yolk absorption when thyroxine and thiourea are administered seems to be slower by about 13% and 7% respectively. Delayed yolk-sac resorption was noted by Warner (11) in brown trout treated with thiouracil.

Thyroid Gland and Thyroid Histology

Transverse and sagittal sections containing thyroid follicles were examined from fish selected at random from the 1951 experiment. Control fish presented a characteristic thyroid picture (7) with low cuboidal epithelium, and slightly basophilic granular colloid containing small, peripheral vacuoles. On the other hand, thyroxine treated fingerlings (121 days after fertilization) had large follicles lined with low cuboidal or squamous epithelium containing abundant eosinophilic colloid lacking peripheral vacuoles. An overabundance of thyroid hormone is suggested. The thiourea treated fish had hyperplastic, hypertrophied follicles of columnar epithelium with small lumina almost completely devoid of colloid. These changes following treatment with thiourea have already been observed in chum salmon (7) and have been described in detail for other fish by several workers (3, 11).

Melanophores

Strips of skin, taken from preserved fish, were mounted on glass slides for microscopical examination. Thyroxine markedly reduced the pigmentation as observed in the living fish and in the prepared slides. Parr marks were completely absent in this group. On the other hand, thiourea had no apparent effect. Reduction in pigmentation following thyroxine treatment has now been observed by several workers and described in detail (8).

Guanine

No guanine was noted on the control embryos prior to hatching. In 1950 guanine deposition in this group of fish commenced 52 days after fertilization. An accelerated deposition then followed on the operculum, body wall growing ventrally around the yolk sac, on upper and lower body wall above the yolk sac, and finally under the pericardial sac. The process was complete about 90 days after fertilization.

In thyroxine treated eggs, guanine deposition commenced on the body wall growing around the yolk sac, about 40 days after fertilization. This is some 12 days sooner than in the controls. Thus newly hatched alevins had an abundant deposit of guanine, as shown in the photograph (Fig. 2).

Although guanine commenced to be deposited in thiourea treated alevins at the same time as in the controls, the 'silvering' was still incomplete on the dorsal body wall, around the pericardial sac and near the anal fin at 101 days after fertilization. On the other hand, when alevins developing in 0.1% thiourea solution were transferred, 84 days after fertilization into 1 : 12,500,000 thyroxine sodium, a striking acceleration in guanine deposition occurred, so that within 17 days of thyroxine treatment a complete silvering had taken place.

Similarly in 1951 experiments, although 'silvering' did not occur until several weeks after the commencement of hatching, a notably accelerated guanine deposition was recorded in thyroxine treated fish. This became very pronounced 51 days after hatching commenced (Figs. 5 and 6). Decreased guanine deposition following treatment with thiourea was noted once more.

Heart Rate

The heart rate was studied as an index of general rate of metabolism, in the 1951 experiment. Just before and shortly after hatching, eggs (with chorion removed) or recently hatched alevins were examined in a constant temperature bath. Ten sets of observations were taken from three fish in each treatment, for a period of three minutes. The mean thus represents the random sampling of 30 fish from each treatment group. Statistical analysis revealed that thyroxine sodium (1 : 12,500,000) and thiourea (0.05%) failed to alter the heart rate significantly. The mean heart rate for control, thyroxine treated, and thiourea treated fish were 62.0, 61.3, and 59.8 respectively. The t values for differences between the means of control heart rate (62.0) and the heart rate of the thyroxine treated fish (61.3) was 0.8 and that for control and thiourea treated fish (59.8) was 1.6. Tabled t value at $P = .01$ is 3.35. Thus, there is no significant difference in heart rates.

Discussion

The effect of thyroid preparations on growth and differentiation of teleost fishes has now been studied by a dozen or more different investigators. The literature has been adequately summarized (4, 5, 6, 9). There is general agreement that the thyroid modifies growth and differentiation but the precise mechanisms are no better understood in developing teleosts than they are in other vertebrates, either juvenile or adult. In the present study, the histological findings, changes in pigmentation, and the general differences observed in growth rate were in agreement with those reported by the majority of previous investigators. Several of the details observed may be of interest.

The marked growth of the body wall with a precocious enclosure of the yolk mass was one of the most striking features. This phenomenon has not previously been described. Baumann and Pfister (1) studied the development of rainbow trout incubated in thyroid solutions and described spectacular effects on blood vessels (not noted in this study) but did not comment on the growth of the body wall. Other studies of this general nature have been carried out on embryos or juvenile fish following yolk-sac closure. The chum salmon with its large yolk mass and slow development showed this effect in a particularly striking manner.

Changes in proportion and rate of growth of pectoral fins were marked. Fins other than the pectorals were apparently unaffected by the treatment. Buser and Bougis (2) describes a similar effect in *Gambusia* but refer to experiments by some other workers in which growth of all fins was modified. One can probably conclude that the pectorals are most sensitive to the treatment. This phenomenon may be a part of the same morphogenetic process responsible for the excessive growth of the body wall.

Marked guanine deposition, following thyroid treatment, suggests an acceleration of nucleoprotein metabolism. Several writers have maintained that thyroid hormone increases the mitotic rate particularly in regenerating or rapidly differentiating tissues (9).

These several effects (early closure of the body wall, intense silvering and elongation of fins) shorten the larval yolk-sac stage and hasten the differentiation of adult characteristics. Other workers have described early formation of epidermal structures and appearance of secondary sex characteristics in young fish treated with thyroid (5).

Both thyroxine and thiourea treated fish grow more slowly in length than the controls. Different mechanisms are probably involved in the two cases. It must be remembered that, although thyroid treated embryos do not increase as rapidly in length as the controls, they are growing more rapidly in other ways. Since measurements of the quantity of yolk present did not indicate any great difference in rates of utilization among the groups, it is suggested that the precocious growth of body wall and fins leaves less material for growth in other ways and the fish increase more slowly in length. On the other hand a different mechanism must be responsible for the relatively slow growth in thiourea. Smith *et al.* (10) argue that the depressed growth in thiourea is a toxic effect and does not provide evidence that thyroid hormone is essential for normal growth in fish. Toxic effects of thiourea have been studied in adult *Fundulus* (3). However, the slow growth of thiourea treated chum salmon might just as well be attributed to absence of a hormone which is essential to normal growth and differentiation. In the presence of thiourea (absence of thyroid hormone) growth continues but at a slower rate. It may be of interest that several workers have found decreased oxidative metabolism in juvenile or larval fish treated with thiourea (3, 5).

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THE PERFORMANCE OF THE LAKE TROUT, *SALVELINUS NAMAYCUSH*, AT VARIOUS LEVELS OF TEMPERATURE AND OXYGEN PRESSURE[1]

By E. S. Gibson and F. E. J. Fry

Abstract

The ultimate upper lethal temperature is estimated to be 23.5° C. The temperature for maximum activity is from 15° C.–17° C. Active respiration becomes dependent on the oxygen pressure at approximately two-thirds air saturation. The minimum oxygen pressure for this species is approximately 40 mm. Hg at 20° C. and 60 mm. Hg at 20° C. These data pertain to one- and two-year fish.

Salvelinus namaycush ranks in importance with *S. fontinalis*, the eastern Brook trout, as a game fish in eastern North America and is also a commercial fish in the Great Lakes. However, in contrast to the brook trout for which a substantial body of knowledge exists (e.g. 3), virtually nothing is known of the environmental physiology of the lake trout. The present study is therefore a contribution to fill this gap. In it we have attempted a laboratory definition of the scope for activity afforded to the lake trout by various levels of oxygen and temperature.

Materials and Methods

The fish used in these tests were hatchery stock, one and two years old, obtained from hatcheries operated by the Province of Ontario. The one-year stock had an average weight of 27.7 gm. with a range from 16.4–37.9 gm. The two-year stock had an average weight of 82.8 gm. and ranged from 57.6–120.6 gm. While in the laboratory the fish were fed freshly ground liver ad libitum every day and not less than 12 hr. before each experiment. Prior to any test, except in the tests to determine lethal temperatures, the fish were maintained in well aerated water at the temperature at which the test was to be carried out for at least a week. In the lethal temperature experiments the thermal history was similarly controlled but the acclimation and the test temperatures did not of course coincide in these experiments.

The general plan of experimentation was that described elsewhere as noted below. Lethal limits of temperature were determined according to the method described by Fry, Hart, and Walker (7), who employed a variant of the method in which the time to death is determined for a sample of fish suddenly exposed to a constant lethal level. The lethal baths were shallow tanks about 22 in. square and 6 in. deep, in which the temperature was controlled to $\pm$ 0.1° C. For a recent discussion and application of this method see Brett (1).

Two levels of metabolism, a standard rate which was the lowest rate of oxygen uptake measured for a quiescent fish in the diel cycle, and an active

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Contribution from the Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto, Canada.

rate which was the maximum steady rate of uptake by fish while they were swimming against a current generated in a rotating respiration chamber, were measured as described by Fry and Hart (6), except for one modification. In the present instance the inlet tube inserted into the Erlenmeyer flask, used as the standard metabolism chamber, was left straight so that water entering was discharged to the center, while the sample was drawn off at the periphery. This modification gave better protection against mixing during sampling. Oxygen determinations were made by the unmodified Winkler method.

The flasks used as standard metabolism chambers in the present investigation were blackened to further minimize disturbance of the fish during the course of the experiments.

Marble chips were placed in both standard and active respiration chambers to neutralize respiratory carbon dioxide.

The swimming rate was measured in the same rotating chamber as was used for the measurements of respiration, except that when the chamber was used for this purpose a continuous flow of aerated water was passed through it. This chamber was annular and of square cross section, 6 in. deep with the outer diameter 12 in. and the inner 6 in. The outer wall was glass, the floor and inner wall were metal. The method of estimating the maximum steady speed attained by the fish is described by Fry and Hart (5).

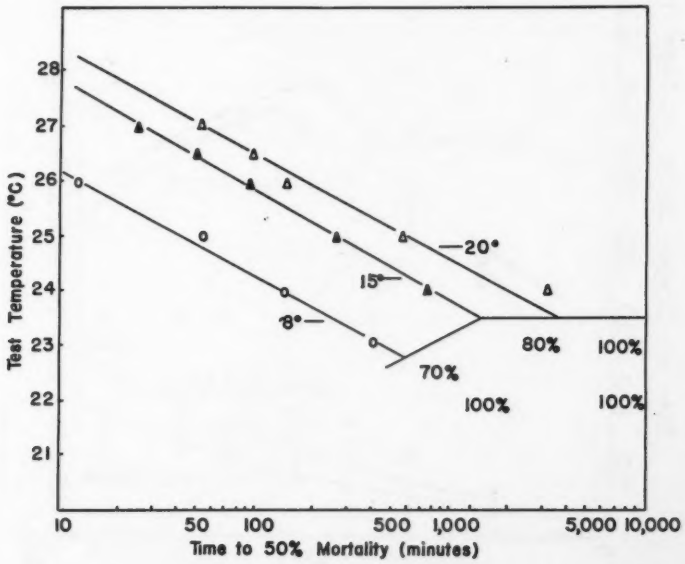

FIG. 1. Lethal temperature relations for the lake trout tested from acclimation temperatures of 8°, 15°, and 20° C. The percentages in the lower right corner indicate the degree of survival of samples exposed to time–temperature conditions represented by the position of the first digit of each percentage value.

Results

Lethal Limits of Temperature

Upper lethal temperatures were determined for samples acclimated to temperatures of 8°, 15°, and 20° C. and the results are plotted in Fig. 1. The points plotted are the geometric mean times to death based on samples of 10 fish at each test temperature. The geometric mean time is equivalent to the median resistance time (7). Each resistance time line terminates at the temperature beyond which 50% mortality does not take place. The oblique and the horizontal line which cut across the bases of the resistance lines together separate the zone of resistance from the zone to tolerance (7). Certain tests were carried out at lower temperatures which aided in the positioning of this boundary. In these more than 50% of the sample survived beyond the expected median mortality time. Such experiments are indicated by percentage survival values. Thus in a test of the 8° acclimated group at 22.5° C., 70% survived for more than 800 min.

The ultimate incipient lethal temperature (7), which is indicated by the horizontal line, is estimated to be 23.5° C.

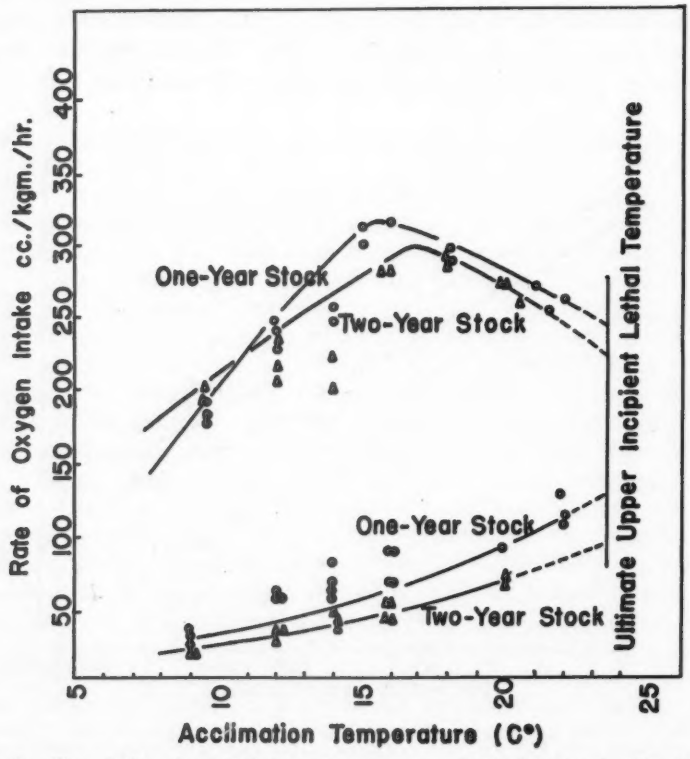

FIG. 2. The relation of metabolic rate to temperature in the lake trout. The lower pair of lines represent determinations of standard metabolism, the upper pair of active.

The Relation of Metabolism to Temperature

The metabolic rate as measured by the rate of oxygen uptake in relation to temperature is shown in Fig. 2. Each point represents one fish in the case of the two-year-olds and three to five fish in the one-year-olds. The lower series of curves represent points obtained under conditions of standard metabolism, the upper series under those of active metabolism. It will be seen that the two series of curves tend to be drawn respectively at the lower and the upper boundaries of the series of points rather than through their means. This has been done on the assumption that the points represent the boundaries of a range of metabolic rates. Thus, in particular, the drop in active rates at 14° C. has been ignored in determining the course of the active curves. The determinations of active metabolism at 14° C. were carried out on the same day on both stocks, hence the divergence of these points from the general trend may have been brought about by something peculiar in the conditions of the experiment on that day.

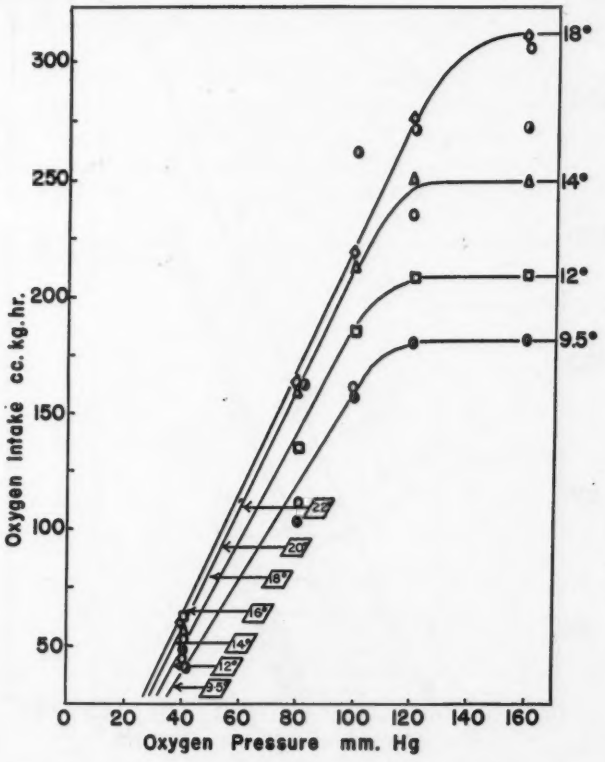

FIG. 3. The relation of the active metabolic rate to oxygen pressure at various temperatures in one-year stock. In addition to the temperatures indicated, points are shown for 16° C. (open circles) and 21° C. (half-closed circles). The arrows indicate the points at which the active metabolic rate is depressed to the level of the standard rate at the temperature indicated.

On the basis of the rate per unit weight the values for the two-year stock tend to fall somewhat under those for the one-year stock. This trend is more consistent in the standard metabolism. Otherwise the course of the curves is similar in the two age groups. The standard curves for both groups display a progressive rise with increasing temperature in the manner commonly recorded for the relation of temperature and standard metabolism. The curves for active metabolism, however, reach a maximum in the region of 15° to 17° C. and then decline steadily over the remainder of the temperature zone this species can tolerate.

The Relation of Oxygen Uptake to Oxygen Pressure

The measures of active metabolic rate were carried out in a closed system in which the oxygen pressure was continuously decreasing in response to the

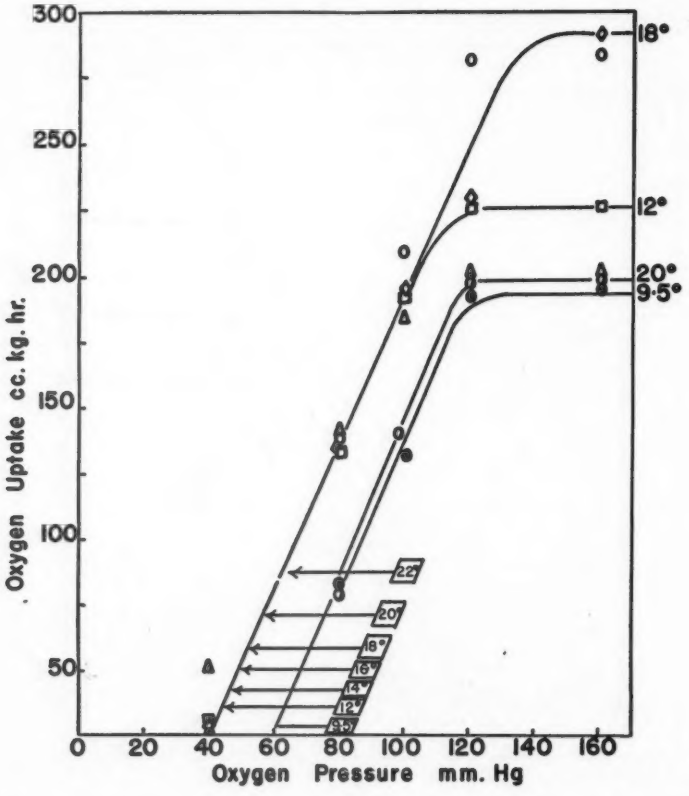

FIG. 4. The relation of the active metabolic rate to oxygen pressure at various temperatures in two-year stock. Triangles indicate 14° C., open circles 16° C. The arrows indicate the points where the active metabolic rate is depressed to the level of the standard rate at the temperature indicated.

oxygen consumption by the fish. Thus the relation of oxygen consumption to oxygen pressure was determined from air saturation down to a low level of oxygen. The rates at various oxygen pressures were determined by drawing tangents to the oxygen consumption curves in the manner described by Graham (9).

The data so obtained are given in Figs. 3 and 4. The active respiration of the lake trout becomes dependent on the oxygen level at a relatively high pressure which is of the order of two-thirds air saturation. The older fish tend to become dependent at a slightly higher pressure than do the younger. The curves at lower temperatures also tend to fall below those at higher temperatures, as has been observed in other species (6, 9). This tendency, however, continues only up to about 14° C. and in the case of the two-year-olds is not well expressed at all; for the 20° C. curve coincides with that for 9.5° C.

The points at which the active rate of oxygen uptake is reduced by diminishing oxygen pressure to the standard metabolic level are also shown in Figs. 3 and 4. These points are designated "the levels of no excess activity" (6), and are taken to indicate, as Lindroth (12) suggested, the minimum oxygen requirement.

The levels of no excess activity thus estimated for the lake trout are plotted in Fig. 5 together with similar determinations for the brook trout. The level increases from approximately 40 mm. Hg at 10° C. to 60 mm. at 22° C. The points for the one-year stock fall somewhat below those for the two-year fish. The lake trout in general is somewhat less sensitive with respect to its

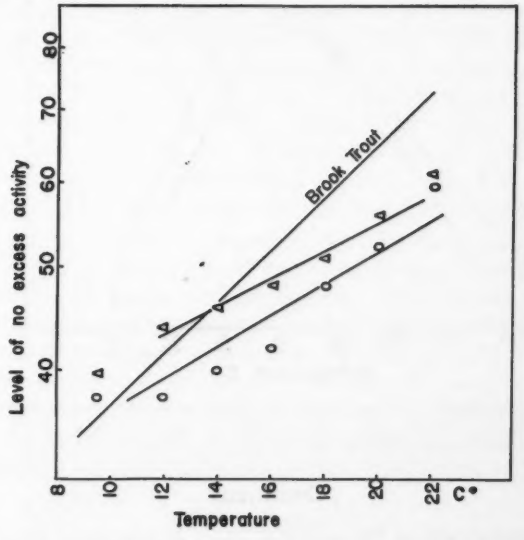

FIG. 5. The estimated minimum oxygen requirements at various temperatures of the lake trout and the brook trout as indicated by the level of no excess activity. Data for the brook trout from Graham (9).

minimum requirements for oxygen than is the brook trout, but the technique by which these determinations were made is probably by no means exact enough to permit any minute comparisons.

Relation of Activity to Temperature

The relation of the steady swimming rate to temperature is shown in Fig. 6. Both age groups display a maximum swimming speed in the region of 16° C. The position of this performance peak corresponds to the region of temperature over which there is the greatest difference between the standard and the active levels of metabolism.

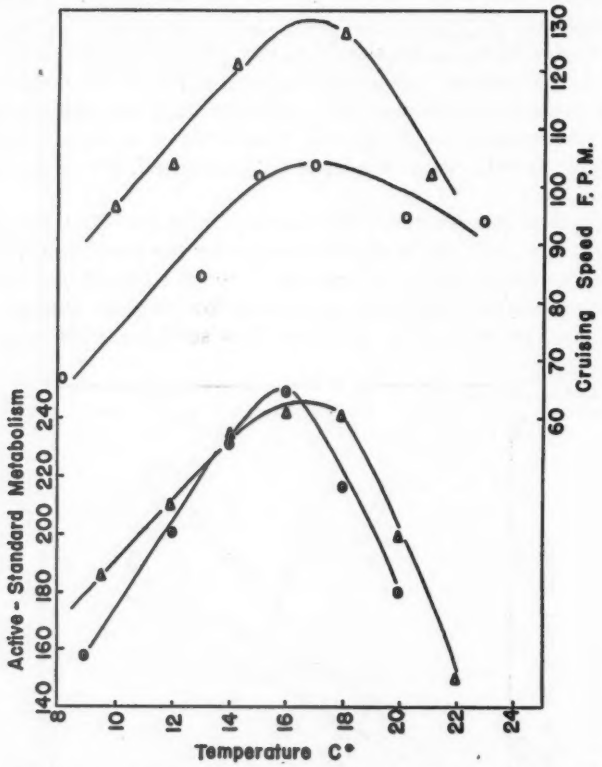

FIG. 6. Upper curves, the relation of the steady swimming speed that lake trout are able to maintain to temperature. Lower curves, metabolism available for physical activity in relation to temperature. Circles one-year stock, triangles two-year stock.

Comment

Salvelinus namaycush is very similar to *S. fontinalis* in its characteristics reported here. The lake trout is, however, somewhat more confined to cold water. Its ultimate upper lethal temperature of 23.5° C. is one of the lowest

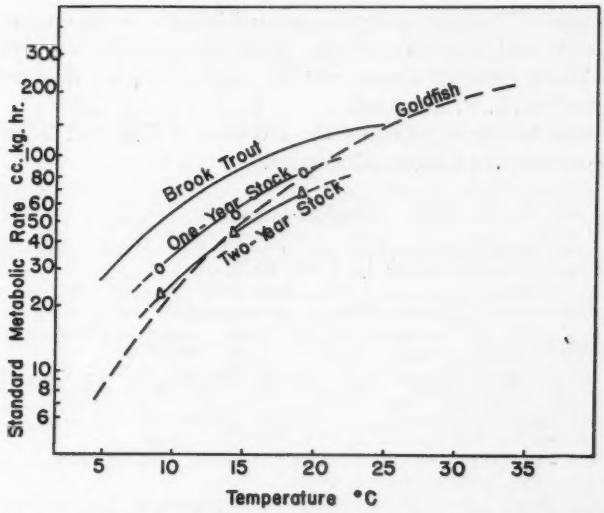

FIG. 7. A comparison of the relative rate of increase of the standard metabolic rate with increasing temperature in two species of *Salvelinus* and the goldfish. Data for the brook trout from Graham (9), for the goldfish from Fry and Hart (6).

on record. Other available determinations for salmonids being *S. fontinalis* 25.3° C. (7), *Salmo fario* 25.3° C. (4), *Oncorhynchus* spp. 23.8–25.1° C. (1), *Salmo salar ca.* 27° C. (2). Like *S. fontinalis*, *S. namaycush* has an activity optimum which is well below its lethal temperature. The peaks for cruising speed, and for the difference between standard and active metabolism, come in the range 14°–18° C. which is at least 7° C. below the lethal temperature. The indications are that *Salvelinus* differs from *Salmo* in this respect. Preliminary work reported by Fry for *Salmo fario* and *S. gairdnerii* and tests by McCrimmon (13) on *S. salar* showed a continuous but gradually decreasing rise in cruising speed right up to the ultimate incipient lethal temperature.

Finally it should be noted that neither the lake trout nor the brook trout follow Krogh's (10, 11) standard curve for the increase of the metabolic rate of poikilotherms with increase in temperature. Fig. 7 shows the behavior of the two salmonids in this respect as compared with that of the goldfish. All three series of measurements were carried out by the same technique. The curve for the goldfish conforms to Krogh's standard curve, which indeed he originally demonstrated by this species. The curves for the salvelinii obviously have a lesser proportional change with temperature although they are not so flat as is the curve for *Salmo fario* as reported by Gardner and King (8).

Acknowledgments

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Maple, Ontario. This laboratory is operated jointly by the Ontario Department of Lands and Forests and the University of Toronto. The special expenses of this investigation were met by a grant from the Research Council of Ontario to Prof. J. R. Dymond.

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AN INTRODUCED POPULATION OF THE GRAY SQUIRREL (*SCIURUS CAROLINENSIS* GMELIN) IN BRITISH COLUMBIA[1]

By D. J. Robinson[2] and I. McT. Cowan

Abstract

A population of gray squirrel (*Sciurus carolinensis leucotis* Gapper) introduced into an isolated forest and park area of 1000 acres is the subject of the study. The squirrels have established themselves in the deciduous and mixed forest areas and in these areas they seem to prevail over the native chickaree (*Tamiasciurus douglasii*) even though the chickaree is physically dominant. The gray squirrel has not adapted itself to new food sources but has merely narrowed its choice until two species, *Acer circinatum* and *A. macrophyllum*, of a genus used on its native range dominate the annual diet. Black color phase predominated over gray (6.5 to 1), the sex ratio was 1 male to 0.645 females, the mean litter size at weaning 1.5. Under these conditions the suitable range is fully stocked and the population is stable. Males roamed over territories of at least 50 acres while females remained within areas of 5 to 15 acres. Thus with polygamy the rule, each male had a theoretical chance of mating with any female. Physical dominance seemed to govern the male actually mating with an oestrous female but the same male did not prevail in each mating observed.

A population of the gray squirrel *Sciurus carolinensis*, firmly established in an isolated area of woodland in the city of Vancouver, British Columbia, presents opportunities for the investigation of the adaptability of the species to ecological conditions not encountered in its native range. It was the purpose of the present study to investigate the adjustments that the squirrel has made to its new habitat, and to explore in a preliminary way the position it has come to occupy in the ecology of the region.

The population under study arose from three or four pairs of squirrels released in Stanley Park, Vancouver, B.C., shortly before 1914. The few original pairs increased rapidly and by 1920 or shortly thereafter, the population had reached a density that has remained fairly constant since that time. Thus the population has maintained an almost constant density for 30 years, as it is effectively prevented from spreading from the park by sea on three sides and by a densely populated city on the fourth. It can be considered that the region is stocked to its maximum carrying capacity.

The origin of the released squirrels is in doubt but it is generally believed that they were obtained in eastern Canada—probably Ontario, and that they represent the subspecies *Sciurus carolinensis leucotis* (Gapper).

The Habitat

Stanley Park occupies a peninsula of 1000 acres. The greatest part of this area is covered with a dense second growth forest of coniferous trees, mostly cedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and grand fir (*Abies grandis*). A small part of the park is developed into cultivated gardens,

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Contribution from the Department of Zoology, University of British Columbia, Vancouver, B.C. Financial assistance was received from the National Research Council of Canada.
[2] *These data are taken from a thesis presented by D. J. Robinson for the M.A. degree in Zoology at the University of British Columbia.*

lawns, aviaries, and pens housing a small zoo. The coniferous area is almost completely uninhabited by the gray squirrel so that the population is confined to the edges of cultivation, and to a fairly extensive area of deciduous and mixed deciduous–conifer forest in the eastern part of the park.

The study was concentrated on an area of maximum squirrel density comprising about 60 acres. The complexity of interspersion characteristic of the area renders it most difficult to give an ecological description of the area that is meaningful for the present purpose. The general nature of the distribution of the four main types of vegetation is shown on Fig. 1, but it should be understood that even in the deciduous association (*Acer–Corylus*) there are large coniferous trees, which offer advantages to the squirrel.

In order to provide an expression of cover structure, random samples were taken of the trees and of the shrub layer.

Tree cover was measured by counting all trunks over 12 ft. in height on randomly selected circular plots 22 yd. in diameter. Each species was then

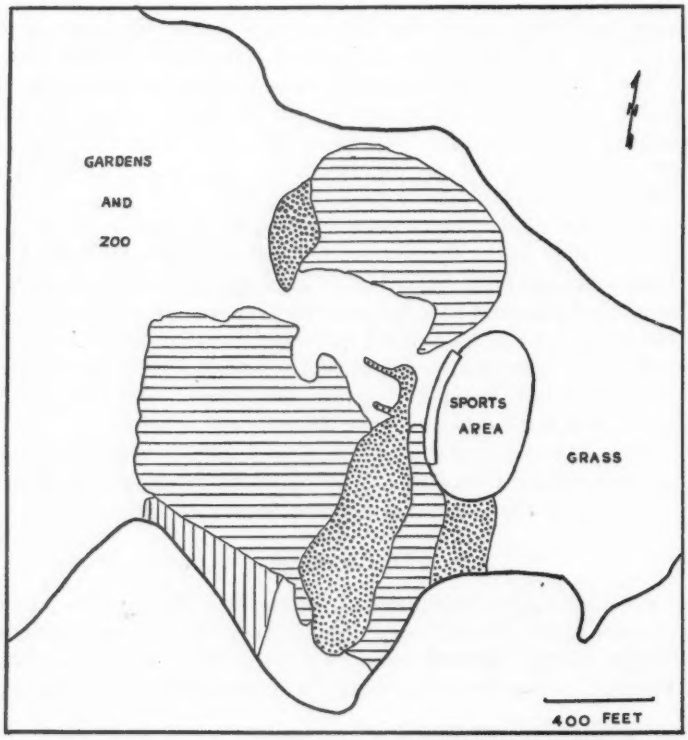

FIG. 1. Distribution of the three forest associations on the experimental area. Horizontal lines = mixed deciduous–conifer type; vertical lines = conifer type; stippling = deciduous type.

expressed as a percentage of the total number of all species. The shrub layer, on the other hand, was tallied by line-intercept method and the percentage expression is that of the amount of ground shaded by each species with reference to the total ground area. While the two methods used do not give comparable results, they were selected as the most satisfactory methods for the purposes of this study.

Table I illustrates the floral composition including all the important species. Obviously a different method of cover appraisal would show different relationships. For example, broad-leaved maple accounts for only 0.6% of the trees, but its growth form with broad, spreading crown gives it a very different importance to a squirrel population than hemlock, which, though more numerous, does not present a similar crown area.

TABLE I

PERCENTAGE COMPOSITION OF THE FLORA OF THE STUDY AREA IN STANLEY PARK, B.C., MAY, 1950

Species	Trees, % over 12 ft.	Shrubs, % under 12 ft.
Vine maple *Acer circinatum*	59.6	3.0
Hazel nut *Corylus californica*	13.5	—
Hemlock *Tsuga heterophylla*	11.4	0.3
Elderberry *Sambucus melanocarpa*	6.3	1.9
Ash *Sorbus aucuparia*	3.0	0.1
Cedar *Thuja plicata*	2.0	0.06
Chokecherry *Prunus emarginata*	1.6	—
Birch *Betula papyrifera*	0.9	—
Cascara *Rhamnus purshiana*	0.6	—
Broadleaf maple *Acer macrophyllum*	0.6	—
Spruce *Picea sitchensis*	0.2	—
Horse chestnut *Aesculus hippocastanum*	0.1	—
Willow *Salix* sp.	0.1	—
Fir *Pseudotsuga menziesii*	0.1	4.1
Salmonberry *Rubus spectabilis*	—	15.3
Huckleberry *Vaccinium parvifolium*	—	1.8
Thimbleberry *Rubus parviflorus*	—	0.5
Foxglove *Digitalis purpurea*	—	0.2
Fern *Polystichum* sp.	—	0.6

It was found that the squirrels moved so widely over the entire study area that it was best to treat it as a single vegetational unit rather than to attempt description of the three subtypes separately.

Both the coniferous association of Thuja–Tsuga, dominant in the coniferous type, and the Acer–Corylus association are regarded as climaxes in response to local edaphic conditions.

It will be noted that *Acer circinatum* and *Corylus californica* are the most abundant species in the area as a whole. Both species produce food edible by squirrels. The understory, dominated by *Rubus spectabilis*, is typical of mesothermal, humid forest areas of the region.

Climatically the Vancouver area is humid, mesothermal, with rainfall adequate at all seasons. The mean temperature for summer is 63° F. and for the winter 38° F. Summer maxima do not exceed 92° F. and temperatures as high as that are rare and of short duration. Winter minima do not go below 2° F. The mean annual precipitation is 57.38 in., the average number of frost-free days is 215, and the annual number of hours of bright sunshine is 1832.

This is not a very different climate than that encountered toward the northern edge of the normal range of the species where in southern Ontario, represented by Toronto, climatic data are as follows: mean summer temperature 64.6° F., mean winter temperature, 25.3° F. Summer maximum and winter minimum 105° and − 26° respectively. The mean annual precipitation is 32.18; the frost-free period, 165 days; and the number of annual hours of bright sunlight, 2048. In general, the Vancouver climate differs in having less extreme temperatures, about 10% less bright sunshine and 70% more precipitation. The first of these differences can be thought of as probably beneficial to the species, the last as possibly detrimental.

Habitat Preference

A preliminary survey revealed that in pure stands of coniferous trees gray squirrels were practically absent. The few that did occur were either transients or dependent upon some abnormal food supply such as scraps of human food. They could not be regarded as members of a normally existing population. As already stated three floral types were represented in the area chosen for intensive study but the ranging propensities of the squirrels made it practical to lump the divisions together for purpose of over-all floral analysis.

However, to evaluate the use accorded to each of these types the average number of animals seen per hour in each during all observation periods was recorded. The results are shown on Fig. 2, and reveal the concentration of activity in the mixed and deciduous woodland. Even less use of the conifer area would have occurred except that in one place a conifer stand separated an attrative nesting and den site from a rich food supply.

The maple groves serve as primary food sources the year round but because of their lack of cover and den sites they have only a limited capacity for resident squirrels.

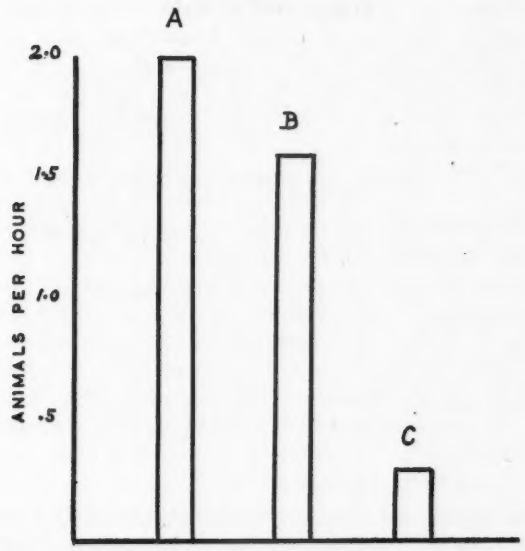

FIG. 2. Number of animals seen per hour in 128 hr. of observation in the three forest types. *A*. Deciduous association, *B*. Mixed deciduous–conifer association, *C*. Conifer association.

The mixed deciduous–conifer habitat, on the other hand, provides necessities other than food, that is den sites, outside leaf-nest sites, cover the year round, and resting stations. The ideal situation would present areas of mixed forest and stands of maple so positioned that both types could be reached within a squirrel's normal cruising radius.

Spatial Relations

In our study of spatial relations, we employed live trapping and systematic observation of marked animals to supply the information on seasonal movement and home range. To facilitate accurate recording of observations, the entire study area was surveyed into ½ acre blocks and the corners of each block marked for ready identification. It was thus possible to locate each observation precisely and to record it on a corresponding grid sheet.

After many experiments with various dyes, which were unsuccessful largely because a large proportion of the population is of the black-color phase, it was necessary to resort to marking different parts by bleaching them with concentrated hydrogen peroxide. This marking was supplemented by clipping different parts of the tail fringe. A combination of these bleaching and clipping techniques made it possible to mark animals for quick identification. There were, in addition, certain animals with distinctive peculiarities by which they could be identified.

Daily Movements

The extent of the daily movements was found to depend upon sex and age as well as upon the extrinsic factors of weather, season, food, and shelter.

In Table II is presented a series of observations of the range of movement of 10 female squirrels, and 10 male squirrels during the winter season and during the remainder of the year. Each entry represents one day's observation of the individual concerned. From this table it will be seen that there is a fundamental difference between the daily movements of the two sexes and that this differs seasonally. Thus in the winter months there was little difference between the range of daily movement of males and females (a mean of 771 ft. as against 839 ft.), if anything the females roamed farther than the males. During the remainder of the year, however, the males moved almost twice as far as the females (1360 ft. per day as against 765 ft. per day). Thus on June 9, a male moved through a radius of at least 2000 ft. during one and a half hours. The maximum recorded movement for any adult female was 1200 ft. from the den tree. Juveniles were never seen more than 300 ft. from the nest until their time of dispersal.

Females with young were observed to have approximately the same radius of daily activity as they utilized the rest of the year. Perhaps then, the female's radius of activity represents the radius of the minimum area that can supply all the needs of an individual.

TABLE II

GREATEST DISTANCES FROM DEN TREE OF MARKED MALE AND FEMALE SQUIRRELS IN A SINGLE DAY'S ACTIVITIES

Female		Male	
Winter	Remainder of year	Winter	Remainder of year
600 ft.	500 ft.	400 ft.	2000 ft.
500 "	600 "	1000 "	1600 "
1000 "	550 "	1100 "	1100 "
1000 "	1200 "	1200 "	1200 "
950 "	1100 "	800 "	1300 "
1200 "	1200 "	400 "	1000 "
500 "	400 "	550 "	1200 "
800 "	400 "	—	1000 "
1000 "	1000 "	—	1200 "
	700 "	—	2000 "
Average 839 "	765 "	771 "	1360 "

While the area utilized by an established female was fairly constant the portions emphasized varied with the season.

The seasonal variation in food supply alone requires that the pattern of daily activity change but several other extrinsic factors also have their influence on the activity pattern.

Seasonal Movements

The seasonal movements observed were those attendant upon the availability of food. Thus during the summer, when vine maple keys are a main source, the squirrels congregate to feed in the groves of those trees. In the winter, on the other hand, the broad-leaved maple is preferred and a larger proportion of the day is spent foraging on the ground.

The only other seasonal change in movement is that already commented upon. The males during the reproductive season roam over twice as large an area as that used during the remainder of the year.

Home Range

For the purpose of this study home range is defined as the area an animal traverses in the normal activities of food gathering, mating, caring for the young, nest and shelter construction, and the other activities necessary for a successful livelihood. The term is restricted to established individuals. The term territorialism is reserved for defense of some area from other individuals of the population. For some species, for example the native Douglas chickaree, *Tamiasciurus douglasii*, the two concepts are largely synonymous but for the gray squirrel there exists a marked distinction.

The study plot had an area of just under 50 acres and supported a population of about 45 squirrels. It would thus appear that there was nearly one squirrel per acre. Such an interpretation would be relatively meaningless. For even within an area so small as this one, the amount of habitat suitable for gray squirrels is considerably less than 50 acres and the suitable areas were, for various reasons, not always available to them. For these reasons the suitable areas supported populations in excess of one per acre.

The largest part of the study area was common ground and the home range of every individual overlapped that of one or several others. This was as true for the males as for the females.

Burt (5) working on small mammals in Michigan, found that the size of the home ranges of the sexes may differ appreciably. This we substantiate for the gray squirrel. The expression of home ranges on a meaningful comparative basis is difficult. The methods used by Burt (5) and by Hayne (16) did not prove applicable to the present study. The most accurate statement we can make on the basis of this study is that males, through the year, moved in a nonrandom manner over the entire study area and thus over at least 50 to 55 acres. Females move similarly through an area of 5 to 15 acres.

Very little truly territorial behavior was exhibited. There was in fact a communal type of association with a high level of intraspecific tolerance.

Groups of from 3 to 10 squirrels were seen foraging on the ground or in vine maples within a radius of 20 ft. Even nests are frequently placed close together. In one instance four nests occupied by different individuals were in adjacent hemlock trees in a grove.

Behavior that can be regarded as territorial was associated with the adult females. These defended the tree in which the nest was placed and vigorously attacked any female entering the nest tree. Evidence suggests that this territorial defense extends beyond the reproductive season as individual females were observed to defend the same nest tree in May, August, and December.

Other types of aggressive behavior of a quasi territorial nature involved the attacking of juveniles by the adult animals during the time of dispersal, and the appropriation of a particularly choice food tree by an aggressive male.

In general, however, intraspecific relations were based upon the establishment of a dominance order similar to that reported for chipmunks (*Eutamias*) (14) rather than on territoriality with the exclusive use of an area that it connotes.

Food

The introduction of an animal into a new environment always involves adaptation of behavior to foods new in nature and of different seasonal availability. Thus the study of the foods used in the new region and their relative importance in the diet may provide important evidence of the inherent adaptability of a species.

In the present instance it was not desirable to kill the animals for stomach analysis, in any event stomach analyses upon squirrels are an unrewarding and unreliable source of accurate information on the diet. Quantitative data were obtained on a basis of animal minutes (11, 8) as used in the studying of the food habits of the larger ungulates. The method has its disadvantages, chief among these is the possibility that nocturnal, and therefore unobserved, feeding will be on a different source (7); that time spent foraging for or eating different types of foods is not always in direct relation with dietary contribution; and that it may be more difficult to observe feeding upon some kinds of foods than on others. However our experience with the gray squirrel leads us to conclude that these difficulties are at a minimum in that species. The gray squirrel is exclusively a diurnal feeder, the population under study was relatively tame and easy to approach, and as a rule a meal is made up principally of one or two food items. It was further observed that there appeared to be little difference between different kinds of food in the time needed to satisfy the appetite.

A few of the squirrels depended almost entirely on "hand-outs" from park visitors and on our 60 acre quadrat about a third of the squirrels made some use of this artificial food source. However, as with our other observations the data on foods and feeding were gathered through a long series of observations made from randomly selected points on the quadrat. In this way it is believed that the data represent the diet of the population accurately.

The separation of the diet into seasonal periods was based on the regional phenology. This gives a more natural grouping of food sources than does dividing the year on calendar months. Table III presents the four phenological seasons and the criteria for their separation.

TABLE III

PHENOLOGICAL SEASONS IN STANLEY PARK, B.C.

Season	Length in weeks	Criteria
Winter: Oct. 30–March 20	18–19	After the shedding of leaves and seed crops and before the squirrels begin "budding" in the spring
Spring: March 20–June 1	9–10	Period of bud growth and shoot development. Terminated by appearance of early mast crop
Summer: June 1–Aug. 23	10–12	Period of utilization of mast crop without storing
Autumn: Aug. 23–Oct. 30	10–11	Period of storage of mast crop. Terminates with dropping of leaves and mast from deciduous trees

On Table IV is presented a summary of the data on seasonal foods and a compilation of the annual contribution to the diet of the squirrels by the various food sources. It is most noteworthy that of the varied food sources available by far the largest part of the diet was contributed by at most three or four species.

The spring diet was the most varied and consisted almost entirely of the buds of trees with *Acer circinatum* and *Quercus* sp. together contributing 74%. The change from the winter diet to that of the spring is a somewhat gradual one at first but as soon as the *A. circinatum* buds began to swell and burst there was an abrupt change in feeding behavior that concentrated activity in the maple groves. The use of *Quercus* was another example of an evident choice. There are only a few large trees in the region but on May 10, after several days of concentration on the maple, the squirrels turned their attention to the oaks and from five to seven squirrels were constantly in attendance in these trees for the next 10 days. It is evident then that very pronounced differences in palatability exist between the species and that the palatability changes abruptly during the different stages of bud growth, leafage, and fruiting.

Though care was exercised to achieve uniformly periodic sampling, it is possible under circumstances of abrupt changes in food preference, such as is characteristic of the spring period, to have some bias occur. In the present study the only doubt in this connection concerns the use of buds of *Acer macrophyllum*. The buds of this tree developed early and rapidly and may have been subject to a short period of intensive use that escaped observation. However, such would make only slight difference in the seasonal picture.

TABLE IV

FOODS OF THE GRAY SQUIRREL

Species	Spring (10)			Summer (12)			Autumn (11)			Winter (18)			Year		
	Parts eaten	Animal min.	% composition	Parts eaten	Animal min.	% composition	Parts eaten	Animal min.	% composition	Parts eaten	Animal min.	% composition	Parts eaten	Weighted forage units	% of total forage units
Vine maple	Buds	469	52.2	Leaves and flowers	633	32.4	Samaras	376.5	65.2	Samaras	17.5	2.1	Leaves, etc.	914.8	17.5
Acer circinatum	Flowers														
	Samaras	2	0.2	Samaras	598	29.8									
Oak	Buds	185	20.7							Acorns	2.5	0.3	Buds	207.0	3.8
Quercus sp.	Acorns	4	0.4										Acorns	9.7	0.2
Horse chestnut	Buds	34	3.6				Fruit	4.0	0.7	Fruit	1.0	0.1	Buds	36.0	0.7
Aesculus hippocastanum													Fruits	9.6	0.2
Weeping willow	Buds	14	1.6										Buds	16.0	0.3
Salix babylonica															
Elm	Buds	15	1.7										Buds	17.0	0.3
Ulmus sp.															
Broadleaved maple	Buds	17	1.9	Leaves	18.5	0.9	Samaras	168.5	29.2	Samaras	532.5	64.6	Leaves, etc.	29.8	0.6
Acer macrophyllum	Samaras	1	0.2	Samaras	431.5	21.4							Samaras	1817.4	34.6
Dogwood	Buds	10	1.1				Fruits	20.0	3.5	Fruit	1.0	0.1	Buds	11.0	0.2
Cornus nuttalli	Fruits	4	0.4										Fruit	40.4	0.8
Salmonberry	Buds			Leaves	9.5	0.4							Leaves, etc.	14.8	0.3
Rubus spectabilis	Shoots	9	1.0	Berries	2.5	0.1							Fruits	1.2	
Hazel	Leaves			Leaves, twigs	169.0	8.4							Leaves, etc.	108.8	2.0
Corylus californica	Petals	7	0.8	Fruits	32.5	1.6							Fruits	19.2	0.4

TABLE IV—(*Concluded*)

FOODS OF THE GRAY SQUIRREL—(*Concluded*)

Species	Spring (10)			Summer (12)			Autumn (11)			Winter (18)			Year		
	Parts eaten	Animal min.	% composition	Parts eaten	Animal min.	% composition	Parts eaten	Animal min.	% composition	Parts eaten	Animal min.	% composition	Parts eaten	Weighted forage units	% of total forage units
Chokecherry *Prunus emarginata*				Fruit	23.0	1.1							Fruits	12.1	0.2
Hemlock *Tsuga heterophylla*				New shoots	10.5	0.5							Shoots	6.0	0.1
Red huckleberry *Vaccinium parvifolium*				Fruit	7.0	0.3							Fruits	36.0	0.7
Red alder *Alnus rubra*							Cones	6.0	1.0	Cones	3.0	0.4	Cones	18.6	0.4
Mountain ash *Sorbus aucuparia*							Fruits	1.0	0.2				Fruits	2.2	
White pine *Pinus resinosa*										Seeds	110.0	13.3	Seeds	252.7	4.8
Hawthorn *Crataegus* sp.										Fruit	11.0	1.3	Fruit	24.7	0.5
Grass										Roots	0.5				
Fungi					7.0	0.3									
Miscellaneous			3.0								16.0	1.9		66.1	1.3
Unnatural foods (Peanuts, biscuit, etc.)		100.5	11.2		56.0	2.8		1.0	0.2	Peanuts	131.0	15.9		449.9	8.5
														5237.7	100.6

Summer is a period of food abundance, and of the change from foliage eating to seed and fruit eating. Here again the shift was abrupt. Within two days following June, the emphasis of foraging had shifted from *A. circinatum* foliage to *A. macrophyllum* samaras. These in turn were replaced by the later maturing samaras of *A. circinatum*. During this period the hazel was the only other species to contribute important amounts of food.

The autumn is traditionally the period of abundance of mast upon which the squirrels concentrate both for current needs and for storage. In this environment the mast crop is small as the majority of species produce small seeds, not attractive to animals as large as a gray squirrel. During this period the heaviest concentration for food was upon the two species of maple, which together provided 94% of the food eaten; the dogwood and alder provided the only other food of note. Food storage is largely directed to *A. macrophyllum*. In this species the samaras are large and occur in groups of a dozen or more that are cut down and handled as units. The hazel does not assume greater importance because it is, in this locality, a very sparse fruiter.

Winter is a crucial time for the gray squirrel population. It is a time of food scarcity and superimposed upon this is the onset of the breeding season. The squirrels depend for food largely upon the stored maple samaras though the few pines, the only large-seeded conifers available, are an important secondary source of food and unnatural foods are more sought after than at any other season.

In preparing the weighted summary of the annual diet we have used the technique described by Cowan (7), which expresses the value of the annual contribution by each food source more accurately than can be achieved by mere summation of the seasonal results. The seasonal percentage contributions are weighted in relation to the length of the season of use. This annual summary will serve to emphasize the dominant position of the two maples in the economy of the gray squirrels. Together they contribute 75% of the annual food intake. Oak, hazel, and pine are the other natural food sources contributing in excess of 1% of the diet.

Comparative Diet

A comparison of the food taken by squirrels of this introduced population with that reported for the normal range of the species is instructive. Several authors (15, 17, 18, 2) report that the gray squirrel in its native range, and in its introduced range in England, consumes a considerable quantity of animal matter such as insect larvae, eggs and young of birds, and small mammals. Little or no evidence of such activity was discovered in the present study. The situation involving squirrel–bird relations is examined later but in summary it can be stated that neither the evidence of nest loss, nor the reaction of birds to squirrels support the idea of our population as nest predators. Goodrum (13) found that in Texas over 3.5% of the food consisted of insects, mainly lepidopterous larvae.

Large lepidoptera are scarce in the study area but it is possible that the squirrels were taking some insects during periods described by us as leaf foraging. Animals were seen to give leaves careful inspection before eating and they may, in fact, have been hunting for insects but we have no reason to believe this is so.

The foods constituting the staple items of diet in the natural range of the species are absent or scarce in the present area. Two possibilities were open to the squirrel: to adapt itself to completely new sources of food such as the abundant cones of cedar, hemlock, and alder among others; or to concentrate, as far as possible, upon the familiar food types available in the new environment.

TABLE V

A COMPARISON OF FOOD ITEMS OF THE GRAY SQUIRREL IN ITS NATIVE AND BRITISH COLUMBIAN RANGES

Native range		B.C.	
Food item	Position	Food item	Position
Quercus (8 species)	Staple	*Quercus* (1 species)	Aux.*
Juglans (12 species)	"		
Ulmus (3 species)	"		
Morus (1 species)	"		
Carya (7 species)	"		
		Corylus (1 species)	Seasonal
Acer (4 species)	Aux.	*Acer* (2 species)	Staple
Crataegus (many species)	Aux.	*Crataegus* (2 species)	Aux.
Cornus (2 species)	Aux.	*Cornus* (1 species)	Aux.
Prunus (1 species)	Aux.	*Prunus* (1 species)	Aux.
Fungi	Aux.	Fungi	Seasonal
Pinus	—	*Pinus* (1 species)	Seasonal

* *Auxiliary* = *taken in limited quantities, less than available.*
Seasonal = *used in quantity when available.*

Table V outlines the situation pertaining to our population in comparison with the native range (largely from (4)). It includes all the important natural food sources of both regions.

From inspection of Table V it will be seen that this introduced population has resorted to the latter alternative mentioned above. Even with the consequence of a much less varied diet than is normal, this squirrel has concentrated on promoting the less favored genus *Acer* to the position of dominance in its diet, rather than turn to other food sources readily available and indeed used by the chickaree on the same area. It would appear that *Sciurus carolinensis* is irrevocably a squirrel of mast-producing woodlands.

Nesting

In Connecticut (12) two species of conifers, the eastern hemlock (*Tsuga canadensis*) and the white pine (*Pinus strobus*), supply 60% of the nesting sites. Other accounts of the life history of the species confirm this preference

for coniferous trees for shelter. Our population has access to a plentiful supply of large conifers that has permitted the fullest expression of this preference. Almost all nests found were in conifers. Our population also builds fewer outside leaf-nests than those of the parent region and this too is probably the result of an abundance of suitable den sites. On the other hand, some of the outside leaf-nests were in use the year round, rather than just for the summer period as is reported for the eastern population.

Twenty-six leaf-nests were found on the area, the majority of them, 20, were found in large hemlock trees at an average height of 36 ft. from the ground, four others in cedars at an average height of 55 ft., and one each was found in a broad-leaved maple and a Douglas fir.

Preferred locations were in order: close against the main trunk, several feet from the trunk on a large limb, or in the crown of the tree. The nests averaged 16 in. × 14 in. and 9½ in. deep. They were made of materials taken from the immediate vicinity and had an outer matrix, 5–6 in. thick, of interwoven twigs and cedar bark. The lining varied, maple leaves, shredded cedar bark, or hemlock twigs being usual. Even after prolonged rains the interiors remained dry.

As already stated most of the squirrels occupied den trees. Sites were abundant in the large, hollow cedars with the many natural splits that they develop at maturity. Virtually all such cedars not occupied by the chickaree were used by the gray squirrels.

A type of temporary shelter not reported elsewhere was found in use here. These shelters were small platforms constructed on suitable locations on broken stumps or on the sides of cedars where projecting bark offered a base. These platforms were all within 8–15 ft. of the ground and were built up higher on the outer edge than the part against the trunk. The squirrels used these shelters as temporary resting places and were not seen to take food to them for eating.

Population

The determination of the population was an important part of the present study and much time and experimentation were directed towards achieving this end. Suitable census techniques for squirrels have been suggested by many previous workers (3, 6), but for one reason or another these techniques were not suitable to the present circumstances.

Only the Lincoln Index, which is based upon the recording of marked individuals, offered much promise of successful application. The nature of the movement of squirrels rendered this a potentially more reliable technique than it would be with a strongly territorial animal. Populations calculated are given on Table VI.

On the basis of the Lincoln Index sampling, the June population was determined to be 35 (25–43); the July population, 39 (31–54); and that of August, 44 (35–60). These results could be checked by calculations derived from the number of females established on the region (13), the sex ratio, and

TABLE VI

POPULATIONS CALCULATED BY USE OF LINCOLN INDEX

Date	Marked animals present	Marked animals seen	Total seen	Calculated population	Fiducial limits
June	6	41	237	34.7	25–43
July	8	30	146	38.9	31–54
Aug.	9	31	151	43.8	35–60

the number of young weaned by the females. The result of this method of calculation is an August population of 50 squirrels, well within the confidence limits of the figure obtained by samples.

Based upon the mean population caculated by Lincoln Index, the spring density was estimated to be 0.69 squirrels per acre, the autumn density 0.88 per acre for a net summer increase of about 26%.

Sex Ratio

A total of 492 separate observations for which sex was positively established gives a ratio of 1 male to 0.645 females. This calculation involves summer observations only. A difference in torpidity during the winter months makes field observations then an unreliable source of data on sex proportions. As an example, the sex ratio derived from field observations of squirrels active in November, December, and January is 1 to 0.333. The tendency to winter torpor in the females was confirmed by observation upon six captive animals.

Age and Sex Variation

Weight and length measurements were taken of 23 live trapped adults and five juveniles. The numbers are too small to be other than indicative, but the data are presented as mean values on Table VII.

TABLE VII

WEIGHTS AND MEASUREMENTS OF ADULT AND JUVENILE SQUIRRELS

	Adults (June and July)		Juveniles (January)	
	Male	Female	Male	Female
Weight (gm.)	594.2	562.5	512.6	512.6
Total length (mm.)	460.8	439.4	448.9	462.4
Tail length	219.7	213.1	225.6	225.6
Number	14	9	3	2

On the basis of these data it can be said only that weight offers a means of distinguishing adult from juvenile squirrels up to the beginning of the reproductive season. This is a different condition than occurs elsewhere in the range of the species and should be investigated further.

As an example of the amount of weight gain that may take place in the second year of a squirrel's life, a female weighed on June 5, $1\frac{1}{4}$ lb. and on Feb. 3, eight months later, weighed $2\frac{3}{8}$ lb., an increase of almost 100%.

Color Phase

Two color phases, gray and black, are known from various parts of the range of this squirrel. Inasmuch as the proportion of the color phase is known to vary with geographical distribution, it is pertinent to record the ratio in our population. Melanism is not inherited as an all or none phenotype, as there are two color types between the extremes of gray and black. However, if all but pure gray be taken as, in some degree, melanistic the ratio is 1 gray to 6.1 melanistic in 492 observations.

A population of this sort, taking origin from a small stock of unknown genetic constitution, may well be biased. However, both color phases are present and have been present throughout the 35–40 year period of occupancy. If gray color is inherited as a simple dominant over black as suggested by Shorter (19) there has been ample opportunity for the virtual elimination of the black phase. That this has not happened, but rather, that the latter predominate, points to some survival advantage of the blacks leading to a delicate equilibrium in the population.

Reproduction

Data on reproduction in our population of gray squirrels were obtained largely by field observation but were supplemented, in certain aspects, by examination of 33 live trapped animals.

The work of several investigators has shown the adult gray and fox squirrels to be dioestrus (1, 10, 4) capable of producing a litter in the spring and another in midsummer. However it is often difficult to establish that certain individuals do in fact give birth to two litters in a breeding season. In the present study two marked females were definitely known to have had two litters.

By examination it was determined that in September, October, and November the testes are small in size, partially abdominal in position, and testicular activity at a minimum. This gave substance to the observation in the field that some males were seen in pursuit of females in all months except the three named. Males reach breeding condition in January and remain active through the succeeding seven months. In the gray squirrel, as with so many species, it is the length of the female reproductive cycle that governs the breeding period of the species.

Successful copulation was first noted on April 8 but the behavior of squirrels prior to that date and the age of litters seen subsequently lead to the

conclusion that fertile matings occurred as early as the beginning of March. For example, the young of eight litters appeared between June 9 and July 6. According to Allen (1) and Brown and Yeager (4) the young are not seen until some seven to nine weeks after birth. This, with a gestation period of 44 days (5) suggests conceptions leading to these litters between March 3 and April 8. These dates are believed to roughly delimit the period of first oestrus.

The second oestrus was at its height between June 15 and July 18 when six matings were seen. Young from these matings would be born between the end of July and the end of August. This was confirmed by the occurrence, in this period, of a second peak in number of lactating females seen. The first peak was in June. The timing of the two reproduction peaks is the same as that in Britain, but differs from that in Illinois and in Texas where the spring oestrus occurs earlier.

We were able to obtain case histories on only two yearling females. One, a member of an early litter of the preceding year had one litter, in the early period of 1951, the other, born in the late summer of 1950 bore her single litter during the late period of 1951. These observations are in accordance with the generalization expressed elsewhere (10, 4).

The most obvious characteristic of the breeding season is the mating chase. Observers watching 6 to 10 males in pursuit of one female have been impressed by the physical aspects of the episode and the biological details have eluded them.

The following account is drawn from six actual chases observed during this study. From one to three days before oestrus the males begin following the female and attempting to copulate, but all such attempts are avoided. The next phase is that of courtship and it can be measured in hours. As we observed it, the female begins to call and is soon surrounded by several males. In the group one male is dominant. This animal begins to call incessantly at the same time staying close by the female. The subordinate males make frequent attempts to displace the dominant animal. The period of conflict continues for one or two hours and during it the establishment of the dominance order is at the same time as the designation of the potential mate for the female. In all instances the female was seen to mate only with this master male. The vocal accompaniment is sufficient to attract all sexually active males over a large area.

It would be expected that such a system of sexual selection would lead to the complete dominance of one male in all matings taking place in an area as small as ours. However this was not so. In the six chases watched at least three different males established dominance. This would suggest that there were internal rhythms in the male possibly akin to those reported in red deer (9) that lead to changes in the ability of an individual to dominate during any long period of reproductive activity in the species.

The next change in behavior is initiated by the female who, relatively quiet during the vocal phase of courtship, becomes very active, running rapidly through the trees and over the ground followed closely by the dominant male

and by the others at varying distances. All the males emit low grunts audible at 10–15 ft. Males that are close follow by sight, others appeared to follow by scent. There is little or no conflict between the males at this stage. The chase culminates when the female permits the dominant male to copulate. The act lasts from 30 to 60 sec. and is repeated several times in a matter of 15–30 min. During this phase and in the quiescent phase that succeeds it, the dominant male will not permit others to approach the female.

Litter Size

We were unable to establish litter size at birth because the circumstances made killing females for uterine examination unwise, palpation results could not be checked and nests could not be reached for the counting of newborn young. Only three adult females, victims of accidents, were available for examination. They averaged three placental scars per litter.

All litter figures then are based upon counts of weanlings or young as they emerged from the nest. Goodrum (13) and Brown and Yeager (4) found the average litter size on the native range to be between 2.5 and 3 young, and Deanesly and Parkes (10) give 3.6 as the average litter of young in the nest in Britain. In Stanley Park, 16 young were weaned in 11 litters for an average of 1.5 young per litter. The details are given on Table VIII.

TABLE VIII

NUMBER OF YOUNG WEANED PER BREEDING FEMALE

No. of females	Size of 1st litter	Size of 2nd litter	Total young
1	2	2	4
1	2	lost young	2
2	2		4
6	1		6
Total 10			16

This is a very low rate of effective reproduction. The three autopsies we performed suggest litter sizes similar to those reported elsewhere. If this is so, losses must be suffered in the nests and we are unable to suggest any likely cause. On the other hand, Brown and Yeager (4) found a significant association of litter size in the fox squirrel with general food conditions and it may well be that the restricted diet of our squirrels is responsible for the small litters.

As has been mentioned earlier, despite this low fecundity, the autumn population was 26% greater than that of the spring. The population is remaining stable so that even with the small litters it seems to be adjusted to the normal losses, and this can be regarded as the annual natural mortality beyond the weaning stage.

Population Controls

Little quantitative information of value was obtained upon this aspect of the life history of the squirrels. Predators present are mink (*Mustela vison*), screech owl (*Otus asio*), great horned owl (*Bubo virginianus*), goshawk (*Accipiter gentilis*), and feral dogs and cats. The horned owl and goshawk are transients and were seen on few occasions. Feral rats (*R. norvegicus* and *R. rattus*) are abundant.

We found the remains of five squirrels, two killed by automobile, one by cat, one apparently by screech owl, and one by an unknown cause. However, much predation, particularly by such as the horned owl and goshawk, would go unobserved in this habitat.

Several parasites were recovered but none seemed to be seriously incapacitating the hosts. Two species of fleas *Orchopeus nepos* and *Hystrichopsylla spinata* were found. The tick *Ixodes angustus* was common from April to October and sarcoptic mange was widespread. The areas usually attacked by mange were the ears, back of neck, and tail.

Interspecific Competition

Only one other sciurid is present in the area, the small chickaree, *Tamiasciurus douglasii*. In its behavior this species showed marked contrast with the gray squirrel. In the first place it is strongly territorial, being very aggressive in its defence of three situations. Food cache areas are defended throughout the year, the nest-tree is a focal point for territorial defense until the young disperse; and finally preferred seasonal food sources are vigorously defended while in use.

Largely because of the aggressiveness accompanying this territoriality, the chickaree was dominant in all conflicts with the much larger gray squirrel. However the degree of conflict is confined by the very different habitat preference observed. The chickaree was almost restricted to areas of coniferous forest.

The relationship between the two species was of an unusual nature. While the grays always give way when attacked by the chickaree, they do not hesitate to trespass repeatedly. As the grays will be concentrated around any desirable food area and the chickaree cannot be constantly on guard at all points, the larger squirrels in effect make extensive use of the parts of chickaree territories that attract them. Territoriality of the sort displayed by the chickaree is only of real function against species with similar behavior. A more communal competitor can take what it needs merely by force of numbers and without physical conflict. We have the interesting situation then, of the chickaree exerting easy physical dominance while the gray squirrel is probably by weight of numbers passively restricting it to areas to which the latter is not attracted. In other parts of its range the chickaree commonly occurs in a habitat of the type occupied by gray squirrels in Stanley Park.

Relationship with Nesting Birds

Several authors have recorded gray squirrel predation upon nesting birds. Middleton (17) concluded that while eggs and young birds were frequently taken by the squirrel the predatory effect was negligible.

In the present study 26 nests representing seven species of birds were found on the study area and kept under observation. The results are summarized on Table IX.

TABLE IX

NESTING SUCCESS OF SEVEN SPECIES OF BIRDS ON THE GRAY SQUIRREL HABITAT

Species	No. of nests	Successful nests
Mallard, *Anas platyrhynchus*	3	3
Kingbird, *Tyrannus verticalis*	1	1
Creeper, *Certhia familiaris*	1	1
Flicker, *Colaptes cafer*	1	1
Ruffed grouse, *Bonasa umbellus*	3	3
Robin, *Turdus migratorius*	11	6
Olive-backed thrush, *Hylocichla ustulata*	6	3
	26	18

Analysis of the cause of nest destruction in the eight unsuccessful nests revealed that the gray squirrels were responsible in two instances (one thrush and one robin), the chickaree in one instance (robin). The others were lost to miscellaneous hazards.

Bird nests were not sought after by the squirrels, but if a bird placed its nest on or close to one of the frequented travelways it was subject to loss. The gray squirrel then can be classed as a chance predator in this region.

Summary and Discussion

This population of introduced gray squirrels has established itself in an area of mixed deciduous–conifer forest not too dissimilar in structure to some parts of its native range but differing widely in the species of trees and shrubs present, and in the humidity and temperature circumstances. In this area it has chosen nesting conditions roughly equivalent to those provided by its native habitat and similarly shows a preference for dens over leaf-nests and for coniferous trees over deciduous trees for the location of outside nests.

Even in its food selection the gray squirrel has shown a conservatism in that it has concentrated its feeding upon the two or three genera occurring here and in its native habitat and has found no new major food source among the several species indigenous in and peculiar to its new habitat. It has thus

restricted the variety in its diet below that reported for any native area. It is in fact basing its survival almost solely upon two species of maples (*Acer circinatum* and *A. macrophyllm*).

Under these circumstances the gray squirrel is maintaining a prebreeding density of 0.69 animals per acre that rises to 0.88 animals per acre in the autumn. The population is relatively stable and has remained so for about 30 years. Its reproductive success is lower than that reported anywhere in its normal range but is adequate to maintain the population here.

The biological data concerning this population can be summarized as follows:

1. Sex ratio of adults is 1 male to 0.645 females.
2. Mean litter size at weaning is 1.5.
3. Adult females are dioestrous with the first period in March and early April, the second at its peak from mid June to mid July.
4. Males are sexually active from January to the end of August.
5. It is promiscuous in its mating habits with several males competing for each female. Mating is exclusively by animals that establish physical dominance.
6. The species is not territorial except in defense by the female of the tree containing the nest and young.
7. At some season the males moved in a nonrandom manner over the entire study area of 50–55 acres, females over 5–15 acres.
8. During the winter males confined their activities to an area similar to that of the females. But during the reproductive season from January to August they traveled the larger area.
9. The two local species of maple *A. circinatum* and *A. macrophyllum* together provide about 75% of the annual food and dominate each season.
10. There is little evidence for the destruction of birds' nests and young. Predation on birds is merely chance.
11. The native chickaree, *Tamiasciurus douglasii*, is intolerant of the gray squirrel and though dominant in conflict, probably suffers some range restriction through passive dominance by the gray squirrel of food sources attractive to it.

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Manuscripts should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables (each typed on a separate sheet) and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts should be carefully checked before they are submitted; authors will be charged for changes made in the proof that are considered excessive.

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An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

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Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Numerous small tables should be avoided.

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